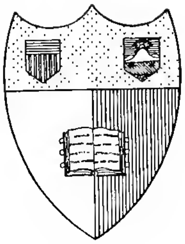

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BACTERIA IN RELATION TO PLANT DISEASES

BY

ERWIN F. SMITH,

In charge of Laboratory of Plant Pathology, Office of Physiology and Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

VOLUME ONE.

METHODS OF WORK AND GENERAL LITERATURE OF BACTERIOLOGY EXCLUSIVE OF PLANT DISEASES.

1902

WASHINGTON, D. C.:
Published by the Carnegie Institution of Washington
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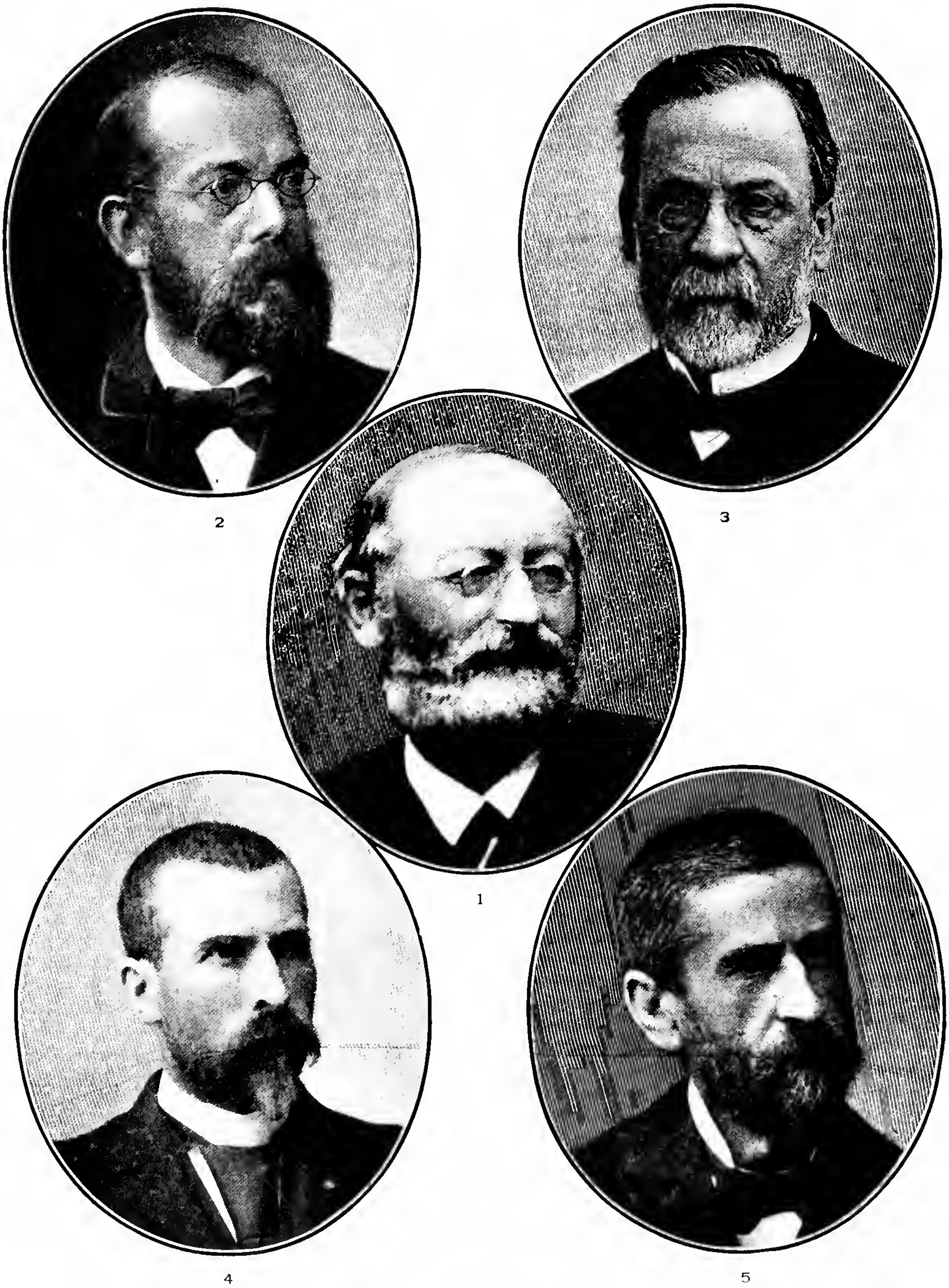
ERIA IN RELATION TO PLANT DISEASES.



1. FERDINAND COHN.
2. ROBERT KOCH.
3. LOUIS PASTEUR.
4. ÉMILE ROUX.
5. ÉMILE DUCLAUX.

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PREFACE.

The subject of bacterial diseases of plants is older than the poured-plate method of Koch, but until recently our knowledge of it has been in a very chaotic state, it having been for the most part for twenty-five years a recognized but uncultivated field. In recent years, however, publications on plant bacteria have multiplied, and they now amount to several hundred titles.

The writer's studies of the bacteria themselves and of the diseases which they cause, as distinct from the literature of the subject, began in 1893. At that time there was very little reliable information on this subject. The literature is now more extensive, but it is nowhere gathered together in one place and properly summarized. It has seemed, therefore, for a long time, that a work of the scope of the treatise here presented might be clarifying and useful to many people. There have been published, and are still appearing, so many papers on the subject of bacterial diseases of plants by writers ignorant of bacteriological methods and indifferent to the requirements of modern pathological inquiry that this whole subject has been brought into disrepute. This is the only possible explanation of the fact that up to a very recent date writers on pathology and bacteriology have been telling their readers that there is no good evidence of the existence of any such diseases.

The following editorial paragraph from the *Botanical Gazette*, February, 1893, may be cited as indicating the general feeling on this subject at that date:

> What is especially needed at this stage of advancement is the continuous and systematic examination of the whole ground by one or more well-equipped investigators, and the publication of a critical statement of what may be safely accepted as proven. Even a summarization of the present status of the subject, without critical laboratory study, would be helpful, if well done.

That this feeling has become intensified with the progress of time and the multiplication of literature is shown by the following citation from the large Treatise on Bacteriology, by Miquel and Cambier, published in 1902:

> The list of bacteria capable of attacking the higher plants increases rapidly from day to day; but whether the experiments of plant pathology offer greater difficulties than those of animal pathology, or whether the authors who have undertaken them have ignored the multiple resources which bacteriology offers to-day, many of the species described must be studied anew, their monography offering regrettable lacunæ. By the side of some fruitful and well-conducted labors we find, unfortunately, altogether too many which must be done over entirely.

It was with the hope of making useful discoveries and clearing up part of this contradiction and uncertainty that the writer began his study of this class of diseases. His first effort in the way of preparation was to supplement his botanical training with a knowledge of bacteriological methods which he obtained from standard literature and competent teachers. His second effort was to gather together and properly digest all of the literature relating to this subject. This resulted in the projection of a critical review of the literature, begun in 1896 in the *American Naturalist* but left unfinished, owing to pressure of research work, and a card catalogue which is now here published in full with critical remarks. His third endeavor was to carefully work over, in the laboratory, field, and greenhouse, as opportunity offered, all of the so-called bacterial diseases of plants, submitting each supposed parasite to all of the tests of modern pathology. The latter has proved a far larger undertaking than was anticipated, the number of diseases attributed to bacteria having increased rapidly in recent years. It is expected that more than 125 diseases will be treated or touched upon in this monograph, many of which have come under the writer's own observation. An attempt has been made to cover the literature of the whole world and to work over personally every disease so far as material could be obtained.

The present volume contains an "outline of methods of work," which was written up in substantially the same form four years ago, in connection with the investigations of the Laboratory of Plant Pathology, Bureau of Plant Industry, United States Department of Agriculture, its publication having been delayed in order to bring the rest of the manuscript into suitable shape. The monograph is published in this form with the approval of the Secretary of Agriculture.

The bibliography at the end of this volume covers the general subject of bacteriology, exclusive of plant diseases, and is arranged chronologically by subjects. Not every good paper is referred to, but for the most part only such as have fallen under the writer's own observation. It is believed, however, that by consulting these the student will soon be able to get hold of the entire literature of any special branch. The reader who wishes to keep pace with the advance of the science should consult the International Catalogue (R) published by the Royal Society of London.

The illustrations, especially those dealing with histology, have been drawn, with very few exceptions, under the direct personal supervision of the writer, every one of them when near completion having been inspected critically and modified in various details to correspond as closely as possible to the actual object. The slides from which the drawings have been made will be placed on file in the Laboratory of Plant Pathology, where they may be consulted.

This monograph is not intended to take the place of ordinary text-books of bacteriology, of which there are now many, but rather to supplement the same, giving information where they are silent or misleading. It is hoped that it will be of value not only to plant pathologists, for whom it is primarily intended, but also to physicians and animal pathologists for purposes of comparison. In its preparation the writer has had also an eye to the service of gardeners, fruit-growers, and all who take an intelligent interest in plants. It is presented with a keen sense of its imperfections, but with the hope that it may at least serve its main purpose. While the writer has made every effort to be accurate in statement and just in criticism, it is too much to hope that he has always succeeded, and, therefore, he desires to crave pardon in advance for all errors of omission and commission, taking shelter behind Lavoisier's well-known and convenient apology: "Man would never give anything to the public if he waited till he had reached the goal of his undertaking, which is ever appearing close at hand and yet ever slipping farther and farther as he draws nearer." Those who dwell in the clearer light of the next generation will build better than we have done and will scarcely realize how slowly and painfully many of us have groped about for what seems to them so plain.

In conclusion, I desire to make grateful mention of Dr. Theobald Smith, professor of comparative pathology in Harvard University Medical School, and Dr. Veranus A. Moore, professor of comparative pathology and bacteriology in Cornell University, each in turn in charge of the animal pathological investigations of the Bureau of Animal Industry, United States Department of Agriculture, at a time when the writer was beginning his bacteriological studies and was perplexed in many ways. To friendly advice and helpful suggestions from these distinguished men he owes more than he can well repay.

AUGUST 25, 1905.

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BACTERIA IN RELATION TO PLANT DISEASES.

BY ERWIN F. SMITH.

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PART I.—AN OUTLINE OF METHODS OF WORK.

GENERAL REMARKS.

The following outline of methods for the study of bacterial diseases of plants, which are now in use in the Laboratory of Plant Pathology, United States Department of Agriculture, has gradually assumed its present shape as a result of the writer's field, hot-house, and laboratory experiments during the past thirteen years. In nearly the same shape, so far as arrangement is concerned, but in a less complete form, it was published in the American Naturalist in 1896.*

The scheme here presented is entirely practicable and is believed to be not more extended than the exigencies of the case require; in the interest of better methods of work in plant pathology it is recommended to all who contemplate a special study of bacterial diseases of plants, and also particularly to those who intend to describe and name species of bacteria, whether pathogenic or nonpathogenic. Those who doubt the necessity for so much work are advised to read procedures recommended for the study of bacteria by a committee of the American Public Health Association, and the earlier paper by H. Marshall Ward (Bibliog., III).† It would be still more to the point if they would isolate a dozen bacterial organisms from the soil, air, or water, and undertake faithfully to identify them by means of any of the older descriptive works, *e. g.*, Eisenberg's Diagnostik or Saccardo's Sylloge Fungorum, or even by such recent manuals as those of Sternberg, Lehmann & Neumann, Flügge, Migula, or Chester (Bibliog., III). Everyone who has carefully inquired into the matter knows that the brief statement of the behavior of an organism on nutrient agar, on gelatin, and on two or three other media, with perhaps a loose statement of its color and size, no longer constitutes a description which describes. Such accounts, of which there are a great many, usually fail to mention just those things which might serve to distinguish the organism from its fellows. If a new species is not to be described so that it can be identified by others, what then is the use of any name or any description? The name will only serve to encumber future synonymy and to recall the incapacity of its author.

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*The bacterial diseases of plants: A critical review of the present state of our knowledge, parts I-VI, Am. Nat., August and September, 1896.

†For Bibliography see end of volume.

THE DISEASE.

The line between disease and health is sometimes a very narrow one, especially when nothing more is involved than some slight change in function. The difference, however, is very striking in many of the diseases here considered. The writer has used the word "disease" in the common acceptation of the term, meaning thereby any marked deviation from the normal functions or structure of the plant as it now exists, whether wild or greatly modified by cultivation. In a sense, such a change as has taken place in the cauliflower, the normal flower-shoots of which have become

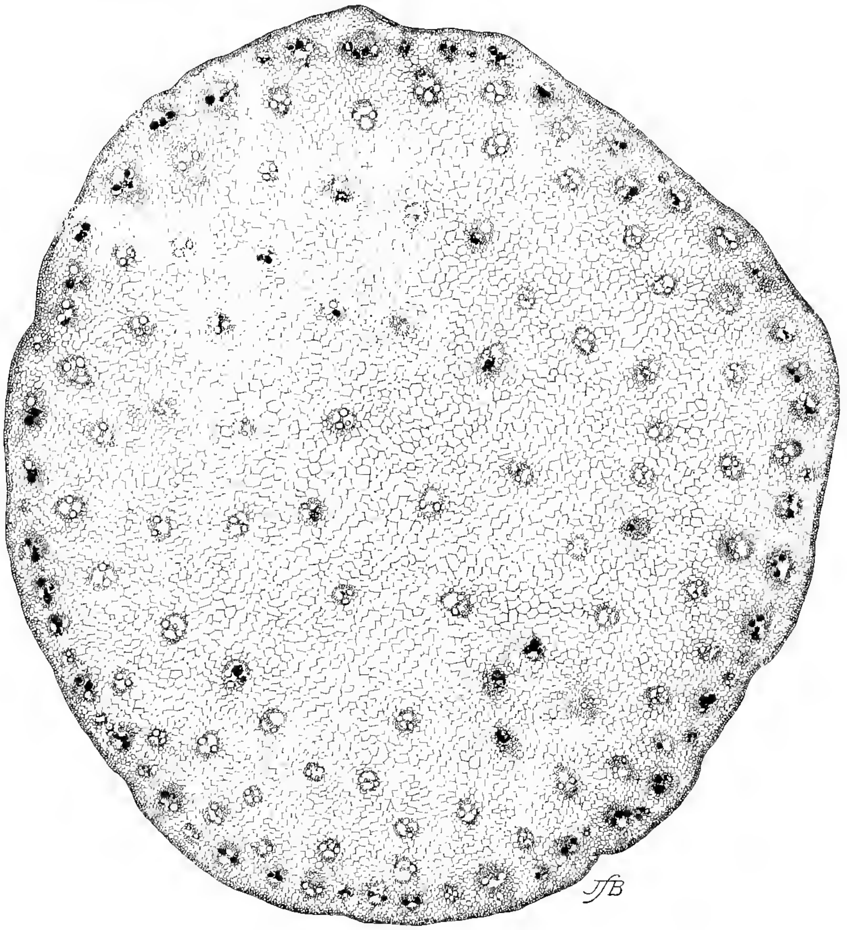

Fig. 1.*

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*Fig. 1.—Cross-section of the upper part of a sweet-corn stem parasitized by *Bacterium Stewarti* (Erw. Sm.). The location of the bacteria is indicated by black shading. Most of the affected bundles are on the periphery. The bacteria have not escaped into the parenchyma. Jamaica, Long Island, N. Y., July 16, 1902. The section was taken several feet from the ground, but the stem infection undoubtedly took place through one or more of the lower nodes. Drawn from photomicrograph of a section stained with carbol-fuchsin. Exactly similar sections, but with a larger number of infected bundles, have been cut from stems of sweet-corn plants infected by the writer in August, 1902, during the seedling stage shown in fig. 73.

compacted, aborted, and enlarged into a fleshy edible mass, might well be regarded as a diseased condition, but it is not so regarded for the purposes of this book. On the contrary, a soft rot of the cauliflower head is regarded as a disease. Bacterial diseases of plants usually involve both functional and structural changes.

Inasmuch as the word "symptoms" has a subjective as well as an objective connotation in medical terminology, the writer has preferred to substitute the word "signs" for those objective characters which serve to distinguish one plant disease from another.

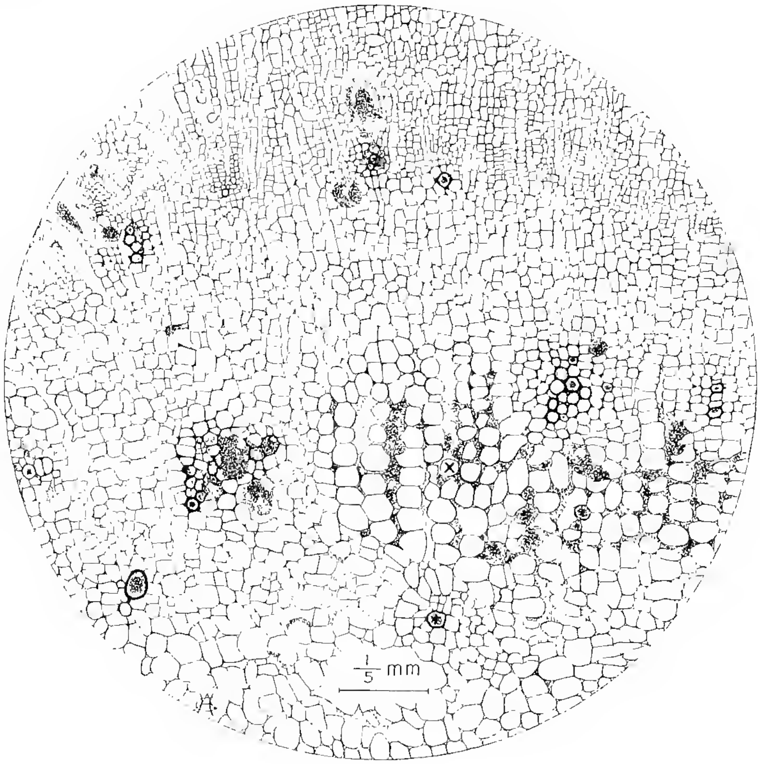

Fig. 2.*

The student will, naturally, first turn his attention to a careful study of the disease. Under this head should be considered: (1) Previous literature; (2) Geographical distribution; (3) Signs of the disease; (4) Pathological histology; (5) Direct-infection experiments.

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* FIG. 2.—Cross-section of a raw carrot, showing wedging apart of parenchyma cells by *Bacillus carotovorus* Jones; from paraffin-infiltrated material. The carrot was fixed in strong alcohol 72 hours after placing on its cut surface one loop of a fluid culture. The inoculation was made in the middle of a cross-section of the whole root, 1 cm. thick, placed in a sterile Petri dish. The surface of the root was sterilized in mercuric chloride water. This section was made several millimeters below the inoculated surface. A small portion of it at X is shown more highly magnified in fig. 3. This section was stained with carbol-fuchsin and bleached in 50 per cent alcohol. Drawn under Zeiss 16 mm. apochromatic objective with No. 4 compensating ocular and the Abbe camera.

In the present state of our knowledge (1) and (2) can usually be considered only after a very careful study of (3), (4), and (5), and of the organism itself. They involve a knowledge of modern languages, and a very considerable familiarity with scientific literature.

PREVIOUS LITERATURE.

One of the first requisites in a student is a knowledge of how to use literature. Previous literature is, however, often of such a fragmentary and uncertain sort, as we shall see, that it is impossible to decide whether a disease is actually new or has been written upon before.

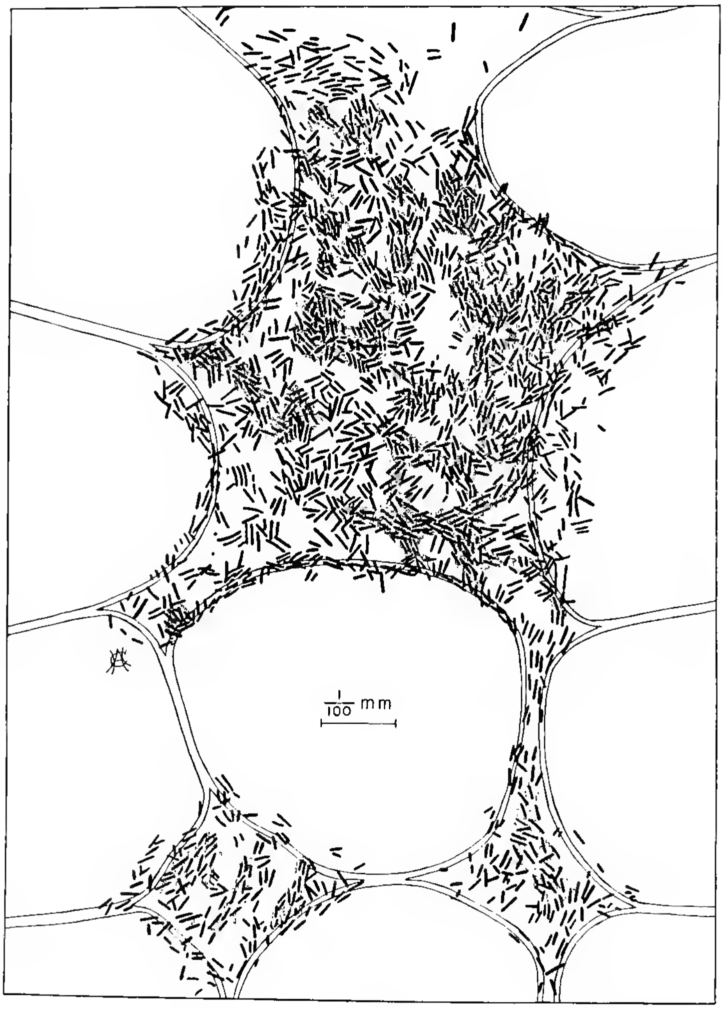

Fig. 3.*

The literature of plant diseases will not be referred to in this volume, except occasionally and incidentally. The bibliography of this volume deals only with general bacteriology—human and animal diseases, methods of work, etc.

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*Fig. 3.—A detail from fig. 2. *Bacillus carotovorus* wedging apart cells of the carrot. Drawn mostly from one plane. In placing the cover-glass a few of the bacteria have been crowded out of the intercellular spaces into parts they did not originally occupy. × 1,000.

GEOGRAPHICAL DISTRIBUTION.

Geographical distribution is an exceedingly interesting problem to many naturalists. The writer shares this feeling and has made every effort to determine it, as far as possible, for each disease. There are, however, still many gaps in our knowledge—the whole subject is so new, and information from all parts of the world is desired. The inner temperature of plants conforms nearly or quite to that of the surrounding medium, and we might therefore expect, in some cases at least, to find a rather more sharply restricted distribution than in diseases of the warm-blooded animals. From theoretical considerations we should expect the distribution of plant diseases to be more like that of diseases of fish and other cold-blooded animals. Whenever the bacterium is able to endure as wide a range of temperature as the host-plant, we should expect to find it as widely distributed.

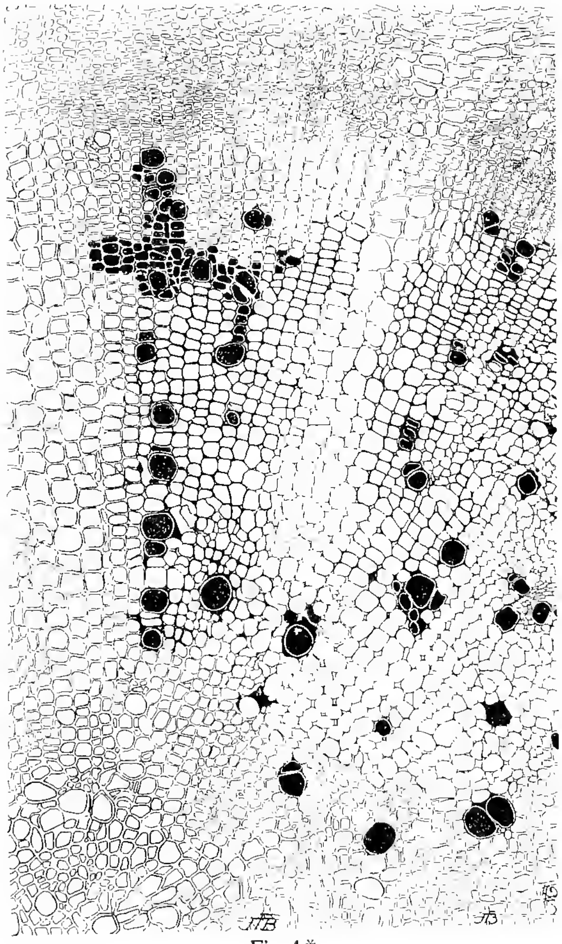

Fig. 4.*

SIGNS OF THE DISEASE.

Great care should be exercised in the description of the physical signs and of the lesions due to the parasite, so that the disease may be identified from these alone, if necessary. A great many cases should be examined and the signs must be recorded in detail and with great accuracy. It should be remembered that here is a frequent opportunity for error to creep in, since the plant may be affected by two distinct diseases which have been confused. Good figures are always desirable, but are not absolutely essential. If possible, however, photographs, pen or pencil drawings, and good water-color sketches should be secured.

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*FIG. 4.—Cross-section of a turnip root, showing vessels occupied by *Bacterium campestre* as the result of a pure-culture inoculation by means of needle-pricks on the leaves. Material fixed in strong alcohol, infiltrated with paraffin, cut on the microtome, stained with safranin-picro-nigrosin, and the differential washing stopped at just the right stage. The bacteria are confined to the vessels and their immediate vicinity. They do not occur in the phloem, a small portion of which is shown at the top of the picture. Section made from the same root as fig. 6, but lower, in the tapering part. Drawn from a photomicrograph. × 85.

When all is said, the signs of many plant diseases, it must be admitted, are much alike, and this is particularly true of the bacterial soft rots. This is an added reason for studying them in each case as critically as possible. The captious reader might also remember that while an enormous amount of painstaking labor has been devoted to animal pathology, including twenty centuries in case of human medicine, we are only in the beginning, so to speak, of our knowledge of the minute pathology of plant diseases, and especially of those due to bacteria.

Pathological Histology.

The relation of the parasite to the tissues of the host should be studied both in fresh material and in stained microtome sections made from material properly fixed and infiltrated with paraffin. The organism may be a wound-parasite, or it may be able to enter through uninjured parts, *i. e.*, in the absence of visible wounds. Often it affects special tissues or systems of tissues. Sometimes the bacteria are quite closely restricted to the vascular system, forming occlusions (figs. 1, 4, 5, 7, and 9). Sometimes they spread widely in the intercellular spaces of the parenchyma, forming numerous cavities (figs. 2, 3, and 6). Sometimes there are striking reactions on the part of the host, *e. g.*, an enormous multiplication of cells resulting in cankers or tumors (plate 2). The habits of the parasite and the behavior of the tissues of the host are best learned from serial sections. The student should not fail to preserve (properly labeled) in strong alcohol an abundance of typical diseased material for future study, exchange, or reference. Stained cover-glass preparations and stained sections should also be mounted in xylol-balsam, carefully labeled, and filed away. Neglect of these precautions prevents the experimenter from furnishing the convincing proofs in case his printed or oral statements are called in question.

As to the best methods of *fixing* plant material containing bacteria much remains to be learned. The writer has had best success with strong alcohol (90 per cent to absolute) and with picric acid dissolved to saturation in absolute alchohol and used boiling hot. In general the watery fixatives can not be used because they do not hold the bacteria in place; even alcohol as strong as 70 per cent allows many kinds of bacteria to diffuse out into the fluid. Boiling absolute alcohol saturated with mercuric chloride is sometimes useful. The alcohol may be boiled in an open Erlenmeyer flask set on wire gauze on an iron tripod over a small Bunsen flame. The alcohol is first brought to a boil. The pieces of tissue are then thrown in and allowed to remain 3 to 5 minutes. It is better to divide the material into pieces suitable for embedding before fixing rather than after. Usually such a piece should not measure more than one-half square centimeter or one-half cubic centimeter. As far as possible only fresh material should be used for this purpose. Old material has often absorbed air in quantity sufficient to render infiltration with paraffin impossible or at least very difficult. In such cases infiltration in vacuo will often render good service. The writer uses a specially devised air-tight paraffin bath connected to the vacuum-pump. Even this device will not in every instance insure perfect infiltration.

DIRECT-INFECTION EXPERIMENTS.

Direct-infection experiments will frequently separate out a parasite which is overwhelmed by some saprophyte and thus furnish better material for plate-cultures, and they are also sometimes very useful when one is remote from laboratories and so situated that it is impossible to obtain pure cultures. It is, however, a crude method and only to be employed when more exact methods can not be used or would not serve as well. By "direct" infection is meant the transfer of fluids or solids from the diseased plant directly into the tissues of the healthy plant, an effort being made to include some of the supposed parasites in this transfer. It is a convenient expression and will be used often in this book.

THE ORGANISM.

This may be considered under three heads—its ability to produce disease, its form, and its physiological peculiarities. Many of the latter might equally well be denominated cultural characters, and the pathogenic properties really belong under physiology, but are kept distinct for sake of convenience and because they constitute not only the most important attributes of the organism, economically speaking, but also a distinct and peculiar phase of the investigation.

PATHOGENESIS.

What constitutes proof of the pathogenic nature of any organism? Upon the ability of the student to give a proper answer to this question depends very largely his success or failure as an investigator. Henle perceived clearly what was necessary as long ago as 1840, and Koch's rules are still fresh in the minds of all. There is consequently now so good an understanding of this subject among animal pathologists and professional bacteriologists that if this book were designed principally for such persons no comment would be necessary. A glance, however, at the literature of plant diseases shows that many of the writers on bacterial diseases of plants have not had this professional training. The four cardinal requirements, as understood by the writer, are as follows:

RULES OF PROOF.

(*a*) Constant association of the organism with the disease.

(*b*) Isolation of the organism from the diseased tissues and careful study of the same in pure cultures on various media.

(*c*) Production of the characteristic signs and lesions of the disease by inoculations from pure cultures into healthy plants.

(*d*) Discovery of the organism in the inoculated, diseased plants, re-isolation of the same, and growth on various media until it is determined beyond doubt that the bacteria in question are identical with the organism which was inoculated.

Under (*a*) there should be numerous observations on many plants, with very careful microscopic examination of stained and unstained material. The cells of many plants contain granules which often dance about so actively (pedesis or Brownian movement) as to be very deceptive, and yet they are not bacteria. Living bacteria in plant tissues can always be stained so as to stand out distinctly if the sections are well prepared and sufficiently thin. When bacteria occur in plants as parasites they are usually very abundant in the vascular system, or the parenchyma, or both, and there is, so far as yet known, always a distinct breaking down (solution) of some portion of the tissues (see figs. 6 and 7, and plate 3). If the parenchymatic tissues are sound, if there is no bacterial ooze on making sections, if the vascular system is not occupied, and if bacteria can not be demonstrated in the tissues by proper staining, then it is very unsafe to infer their existence from dancing particles, no matter how many may be visible in the unstained sections. Moreover, bacteria may be present in some of the plants and not in others, *i. e.*, not constantly present, and so not the cause of the disease. It is conceivable that they might also be present

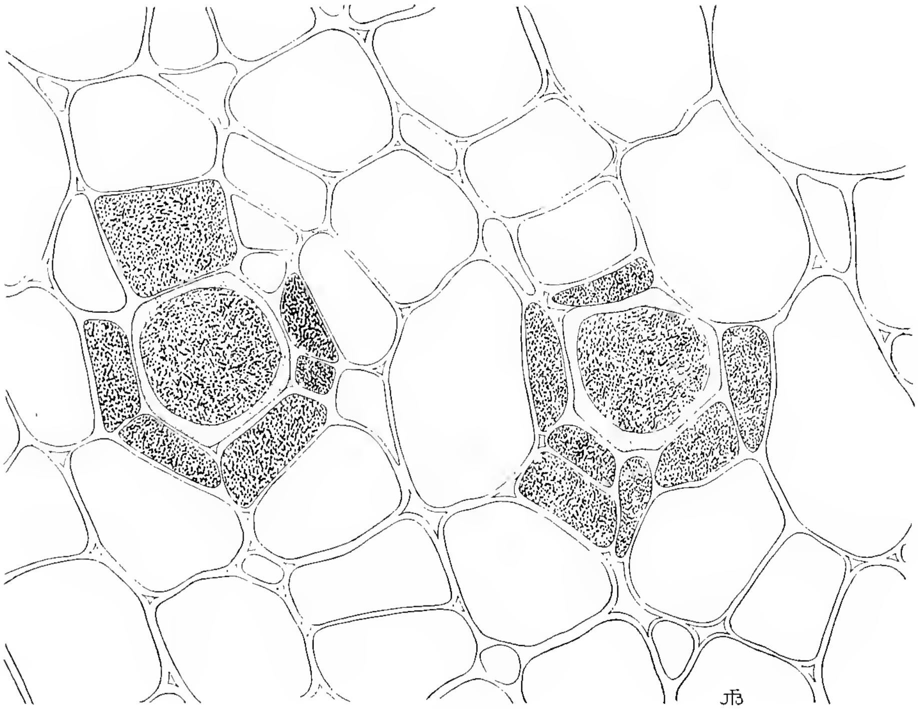

Fig. 5.*

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*Fig. 5.—*Bacterium campestre* parasitic in a turnip-root (inoculated plant No. 53). This figure shows the bacteria crowding out into the cells surrounding the reticulated vessels. The lignified portion of each vessel is indicated by fine dots. Material fixed in strong alcohol, infiltrated with paraffin, cut on the microtome, stained with carbol-fuchsin, and the excess of stain removed in dilute alcohol, section then dehydrated and mounted in xylol-balsam. Drawn from a photomicrograph, the contrast here indicated being not greater than that shown in the section. × 500 circa.

quite constantly, but merely as followers of something else. When possible, therefore, diseased plants should be examined for the suspected pathogen, in large numbers, in different years, and from widely separated localities. Of course, if fungi are also present they must likewise be examined as to constant occurrence and pathogenic properties.

Under (*b*) all of the standard nutrient media should be tried, and that repeatedly, until the student is entirely familiar with the appearance and behavior of the organism. It is usually best to isolate the organism for experiment from selected portions of the tissue by means of Esmarch roll-cultures or by the use of poured plates (Petri-dish cultures), generally the latter.

Isolations may also be made by inserting a sterile platinum needle or loop into the diseased tissue, obtaining therefrom a little fluid, and drawing this over the surface of slant agar, gelatin, or potato a number of times. This is an old method introduced by Koch in 1881. If ten or twelve tubes are used, the final streaks will often consist only of scattering colonies, from one or more of which the subcultures may be made. The plate method has the great advantage of showing just how many kinds of bacteria are present in the tissues (provided they will all grow in the medium used and under the conditions of the experiment), and just how numerous they are. In case of viscid organisms, or those forming compact zooglœæ in the

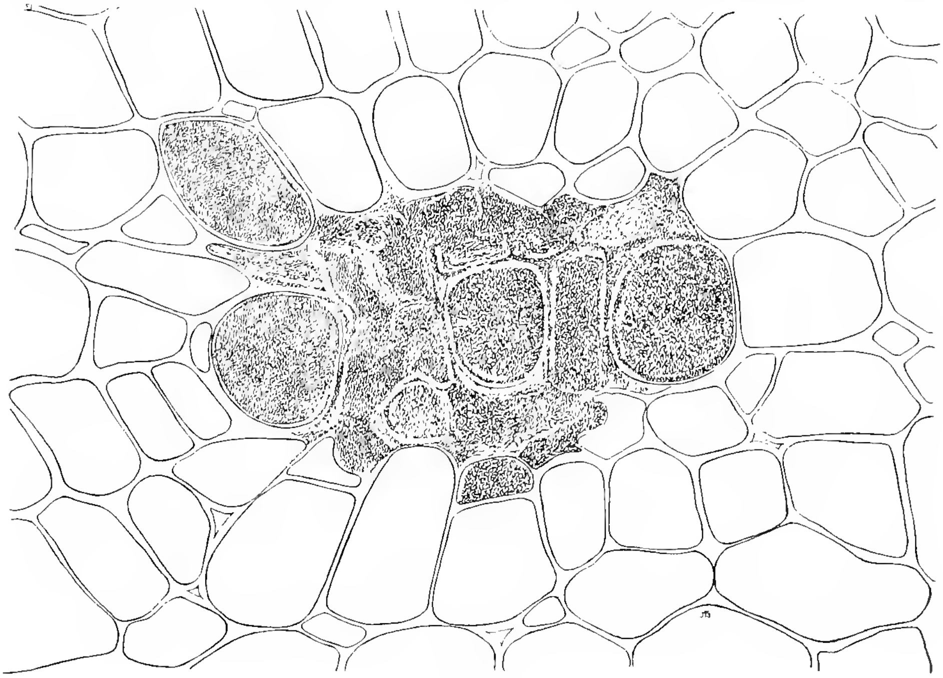

Fig. 6.*

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*Fig. 6.—Cross-section of root of plant No. 53 (turnip) parasitized by *Bacterium campestre*, showing an early stage in the formation of a bacterial cavity. The original section was made from material fixed in alcohol, infiltrated with paraffin, stained with carbol-fuchsin, and washed in a mixture of alcohol and water. Drawn from a photomicrograph. × 500

tissues, it is sometimes desirable to grow them for a day in bouillon before attempting the plate-cultures ; but one must then be on his guard, since it is quite possible by this method to start with enormous numbers of the right organism and have the bouillon culture filled with something else at the end of the 24 hours.

Pure cultures may also sometimes be obtained by cutting out pieces of the tissue and throwing them into tubes of culture media. This method, however, shows little or nothing as to the prevalence of the organism in the tissues, and in the hands of beginners is very liable to miscarry. If growth is obtained it may indeed have come from many organisms of one sort pervading the tissues and causing the disease, but it is not certain that it did not result entirely from one or

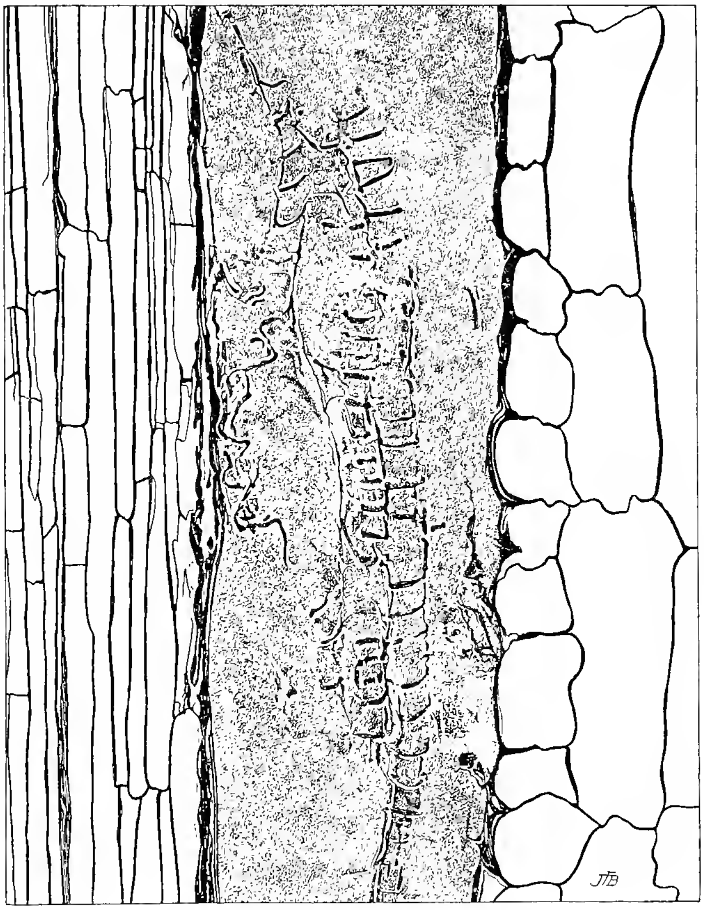

Fig. 7.*

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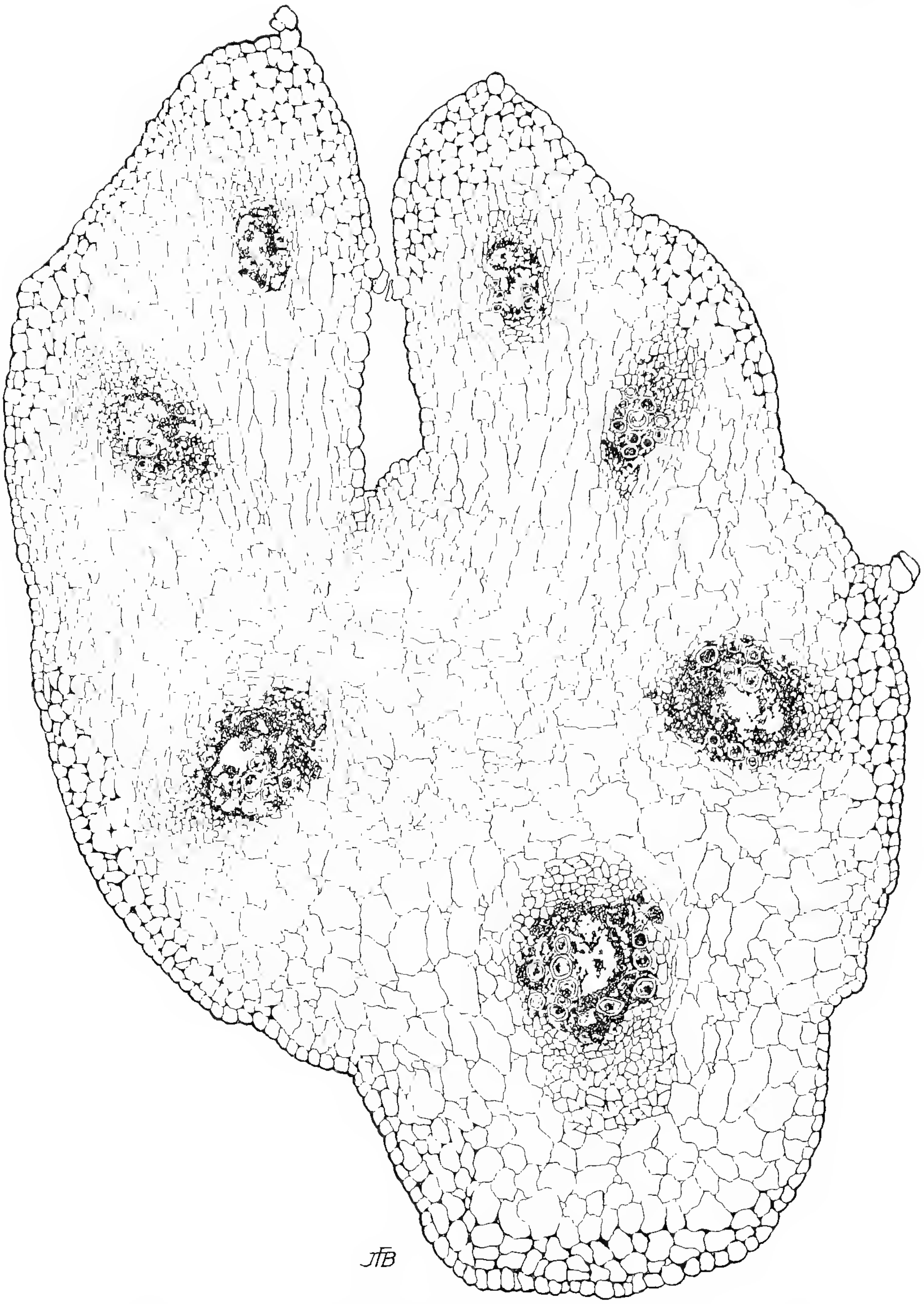
*Fig. 7.—Bundle in a cauliflower-petiole entirely destroyed by *Bacterium campestre*. The result of a pure-culture inoculation. Plant No. 112 inoculated March 10, 1897, by needle-punctures on the blade of a leaf without hypodermic injection. First signs of disease March 20. Petiole put into alcohol on April 5. Longitudinal section. Tissues surrounding the bundle entirely free from bacteria. Section not made from the inoculated leaf, but from the first leaf that showed secondary signs. Drawn from photomicrograph of a paraffin section stained with carbol-fuchsin. × 206.

PLATE 3.



Cross-section of petiole of muskmelon No. 150 attacked by *Bacillus tracheiphilus*.

The bacteria are confined to the bundles, in each of which cavities have appeared. This section was taken from near the point marked X on the inoculated leaf (see fig. 8). The inoculations were made on the blade of the leaf by means of delicate needle-pricks. The material was collected and fixed in strong alcohol on the 6th day after the appearance of the disease.

more bacteria accidentally introduced from the surface of the plant, from one's clothing or body, or from the air; or it may have resulted from a few non-pathogenic organisms accidentally present in the inner tissues of the plant, particularly in case of roots which have been dug some time. It is therefore much better for the student to begin with plate cultures. Generally speaking, the parasite will be more easily obtained in a state of purity from plants or organs of plants recently attacked and from deep tissues, or from just within the margin of advancing diseased areas, rather than from near the surface, or from parts which have been diseased for a considerable time.

Parts long affected almost always contain mixed growths due to the multiplication of saprophytes of various kinds. From such parts it is usually much easier to obtain the saprophyte than the parasite, even if the latter has not been entirely crowded out and destroyed.

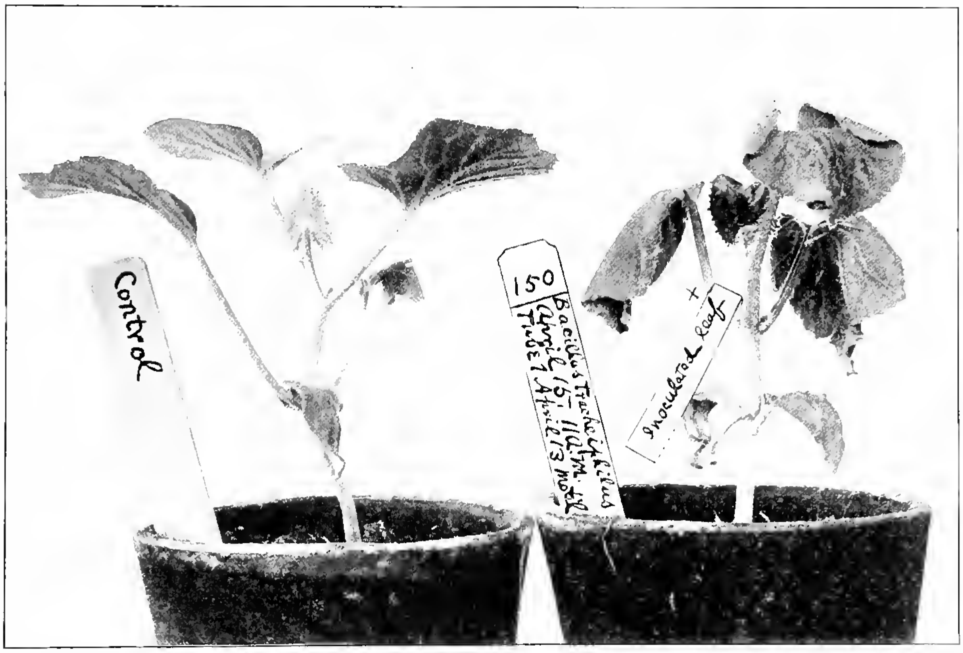


Fig. 8.*

Great care must be exercised to avoid introduction of surface organisms which might complicate results, especially if rapid growers. The easiest and most satisfactory way, when the tissues will admit of such treatment, is to sear the surface with a hot knife or spatula so as to burn all surface organisms and then cut or dig through this sterile surface with hot or cold sterile scissors, scalpels, forceps, or needles to a part which has not been affected by the heat, from which some of the diseased fluids and solids may be removed on a sterile platinum loop. I frequently sear upon sound tissues at one side of the spot from which I desire to make cultures

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*Fig. 8.—Muskmelon plant No. 150, inoculated with a pure culture of *Bacillus tracheiphilus*. The pricked leaf is on the left side. The section shown in plate 3 was taken from the point marked X, three days after the photograph was made and ten full days after the inoculation.

and then dig under into the periphery of the diseased portion. If the tissues are rather dry the bacteria may be forced into the cavity by careful squeezing, or some drops (loops) of sterile water or beef-bouillon may be introduced into the cavity and stirred around before the bacteria are removed. If heat is inadmissible, the specimen may be washed or soaked for a time (15 seconds to 60 minutes) in mercuric chloride water (1:1000) and the surface thus freed from many contaminating organisms. Carbolic acid (5 per cent in water) or lysol (5 per cent in water) may also be used for sterilizing surfaces. Of course these substances must be removed as far as possible before the surface is broken. This may be done to some extent by swabbing with sterile absorbent cotton dipped into sterile water or by plunging into sterile water and shaking. The disinfectants will be more certain to touch and sterilize every part of the surface if all adhering particles of air are driven off by first plunging into alcohol for a moment.

In case of bacterial leaf-spots the writer generally obtains satisfactory cultures by cutting out the spot and plunging it for a few seconds (15 to 45) into 1:1000 mercuric chloride water, then rinsing in sterile water for a few minutes, crushing and throwing into a tube of bouillon from which the plates may be poured in course of an hour, *i. e.*, as soon as the bacteria from the interior of the spot have had time to diffuse into the bouillon. I frequently crush with a sterile glass rod, after throwing the material into a tube of bouillon, or else on a small sterile cover-glass which is then thrown into the bouillon.

In cases where heat and chemical disinfectants are both inadmissible on account of danger of destroying the organisms within delicate tissues, as in thin leaves and other soft parts, the bacteria or fungus-spores accidentally lodged on the surface may be greatly reduced in number by gently rubbing all parts of the surface between the thumb and finger under distilled water and then washing them in three or four successive beakers of distilled sterile water, the fragments being transferred from one beaker to the other by means of sterile forceps. Of course, the thumb and fingers must be well cleaned in advance by scrubbing and sometimes by the use of alcohol and corrosive sublimate, followed by sterile distilled water. When dry, these washed specimens may be scraped into, directly for plate cultures, or after the epidermis has been peeled off with cold sterile knives and forceps.

Quantitative determinations may be made by grinding up a given quantity of the suspected plant tissue, *e. g.*, a cubic centimeter or a gram, in a sterile mortar with clean sterile sand and 10 or 20 cc. of beef-broth or sterile water, and then making plates from carefully measured portions of the fluid, *e. g.*, from one 2-mm. loop, from 0.1 cc., 0.5 cc., etc. A like number of check plates made from equal portions of healthy tissues ground under precisely similar conditions will soon demonstrate about how many colonies are to be expected per plate (and what kind) as the result of surface contamination or air-borne bacteria introduced during the process of grinding.

The procedures described under *c* and *d* should be repeated a number of times (the more the better) and always with uninoculated plants in abundance for comparison. *These control-plants or check-plants must remain healthy.* If they also become diseased, then the experiments must be done over with more care and times enough to remove all possible chance of error. When check-plants become diseased, especially in any number, there is always room for grave suspicion. Either the experimenter has been grossly careless, assuming that he used the right organism in his inoculation-experiment, or else he is working in a locality where the cause of the disease is naturally abundant. In either case, however well convinced he himself may be, his readers will generally have a lingering suspicion that even his inoculated plants succumbed not to what he inserted into them, but to some entirely different cause naturally present and overlooked by the investigator. The remedy for the first is to learn to use infectious material with more caution, and for the second is to make the inoculation-experiments in localities or under conditions where the plant shall be less subject to natural infection.

If the experiments must be performed in localities where the disease is naturally present, then a large number of plants must be selected for inoculation and for control, and such a high percentage of infections secured in the inoculated plants that the few cases occurring naturally in the control-plants may be neglected as not casting any doubt on the general result. For example, if, in a region subject to the given disease, 100 plants were reserved for control and 100 similar plants were inoculated, and out of this number 50 of the latter and 40 of the former should contract the disease, it is manifest that no deductions of any value could be made from the experiment. All might be the result of some cause totally different from the

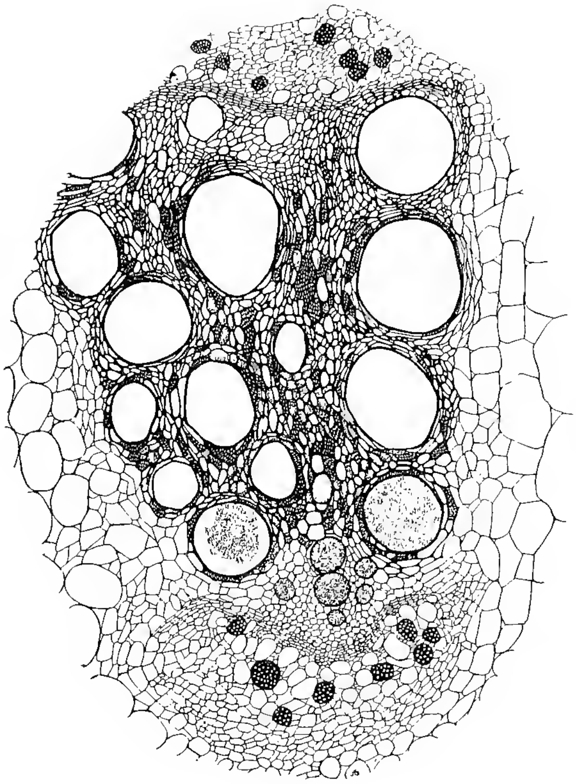

Fig. 9.*

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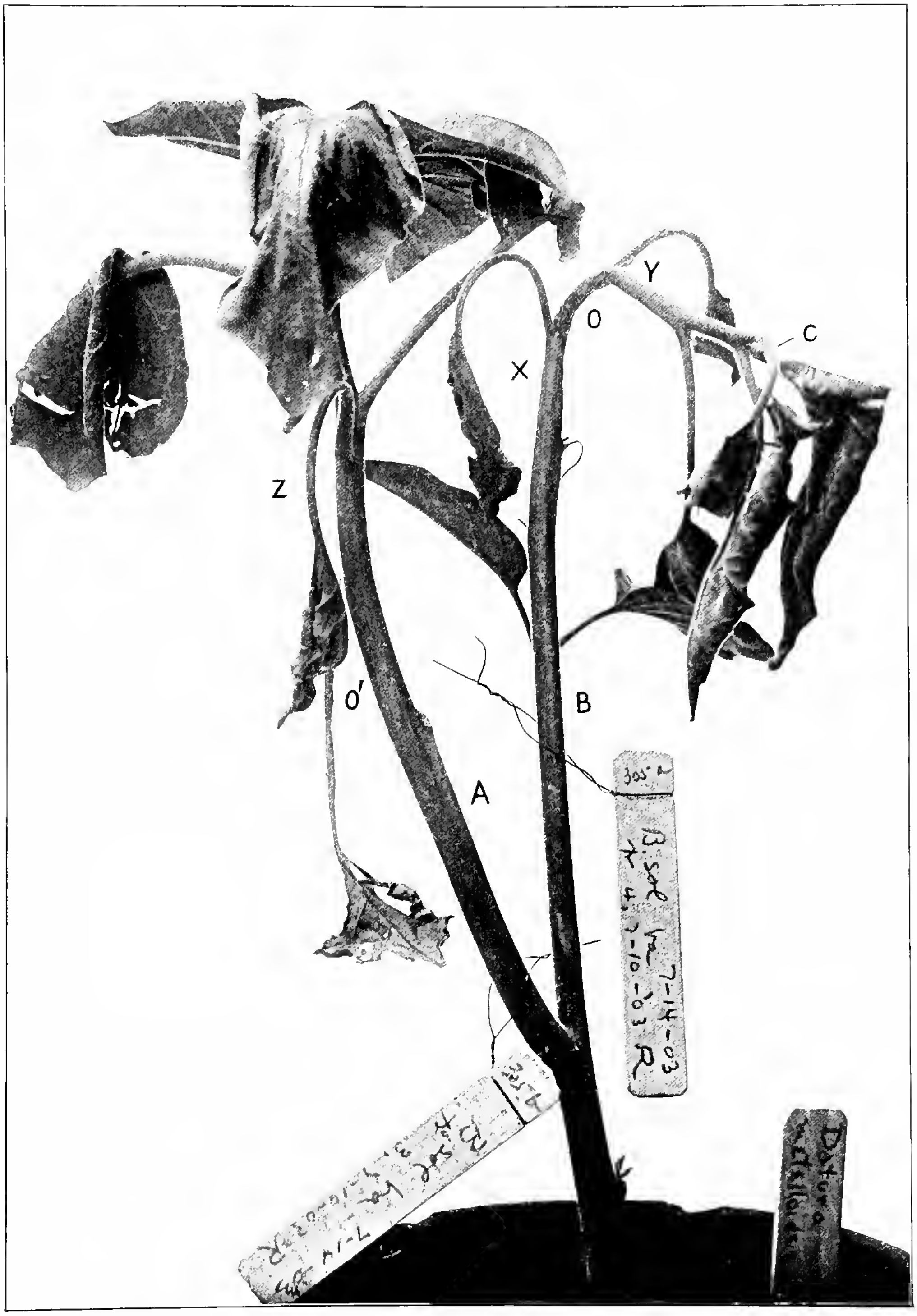
*FIG. 9.—Cross-section of a small part of a cucumber stem, showing the parasitism of *Bacillus tracheiphilus* in one of the inner bundles. As yet there is no bacterial cavity, the bacilli being confined to the spiral vessels and a very few of the adjacent pitted vessels. Material taken from a field near Washington, D. C., in 1893. Sectioned from paraffin. Drawn from a photomicrograph. × 50. Introduced for comparison with plate 3. Beginning at the top, the tissues occur in the following order: (1) Outer phloem, showing sieve plates; (2) cambium; (3) immature xylem; (4) mature xylem, consisting of pitted vessels and pitted connective tissues; (5) spiral vessels embedded in non-lignified living parenchyma, which is finally destroyed by the bacteria; (6) pseudocambial layer; (7) inner phloem; (8) large-celled parenchyma to either side, separating this bundle from its neighbors.

assumed cause, the different number of cases in the two groups of plants being accidental variations. If, in such a locality, only a very few plants are inoculated and a few held as checks, the evidence becomes still weaker and would not be considered entirely conclusive even though all of the inoculated plants should contract the disease and all of the checks should remain free, since in a region subject to a given disease five or six healthy plants may sometimes be found in proximity to five or six diseased ones, although all may have appeared healthy earlier in the season. The case is quite different if out of 100 control-plants and 100 inoculated plants 95 per cent of the latter and only 2, 5, or 10 per cent of the former contract the disease. It then becomes a question of probability which may be converted into reasonable certainty by several repetitions of the experiment with like results. Of course, the ideal experiment is one in which all the inoculated plants contract the disease and none of the control-plants, and in which a large number of plants has been used so as to exclude all possibility of the results being due to anything but the organism used.

Whenever the disease occurs naturally in the vicinity selected for the experiments, too much emphasis can not be laid on the necessity of having numerous inoculated plants and numerous controls, and on the desirability of repetitions of the experiment in different years and under different local conditions. It is important also that the inoculated plants should be under healthful conditions, *i. e.*, under conditions as nearly natural as possible. For example, proper (natural) conditions would be much more nearly attained by inoculating vigorous plants growing in the open air or in well-kept greenhouses than by inoculating parts of the same plants cut away from the stems and kept under bell-jars. It is conceivable that inoculations which would succeed very well under the conditions last named, especially at abnormally high temperatures, might entirely fail when under a more natural environment.

Not one of these four requirements can be omitted safely. A chain of evidence is not stronger than its weakest link. *Particular stress, therefore, is laid on being able to produce at will the characteristic signs and lesions of the disease in healthy plants by inoculation with pure cultures of a given sort; also on the re-isolation of the organism from the artificially-infected plants after they have become diseased, on the subsequent proper behavior of the organism in nutrient media; and on its ability to produce the disease when again inoculated.* This is the whole thing in a nutshell. The experiments must be continued until there is no doubt whatever as to the pathogenic or non-pathogenic properties of the organism. "Almost certainly pathogenic" always leaves room for grave doubt in the mind of every thoughtful reader. As a rule, the re-isolations should be made at a considerable distance from the point of inoculation, particularly if there is any doubt whatever as to the identity of the physical signs, since saprophytes have been known to live in plant tissues for a considerable number of weeks near the place of inoculation, and, if abundant, might cause various disturbances of nutrition without being the pathogenic organism sought for. For example, one would be more likely to obtain the cause of the disease in pure culture by attempting isolations from a plant in the stage shown in

PLATE 4.



Datura metelloides inoculated by needle-pricks with *Bacterium solanacearum* (Erw. Sm.).

The stems were pricked at O and O′ on July 14, 1903, and the photograph was made July 22. The first signs of wilt appeared the 4th day. About one-third natural size.

plate 4 than from the same plant a week later (fig. 10). One would also be more certain of pure cultures by plating from the interior of the plant at A, B, or C, rather than at X or Y.

The judgment of experienced bacteriologists as to the pathogenic nature of an organism may to a certain extent be accepted in absence of full proof, but only for the time being. Nothing is absolutely certain which has not been experimentally demonstrated.

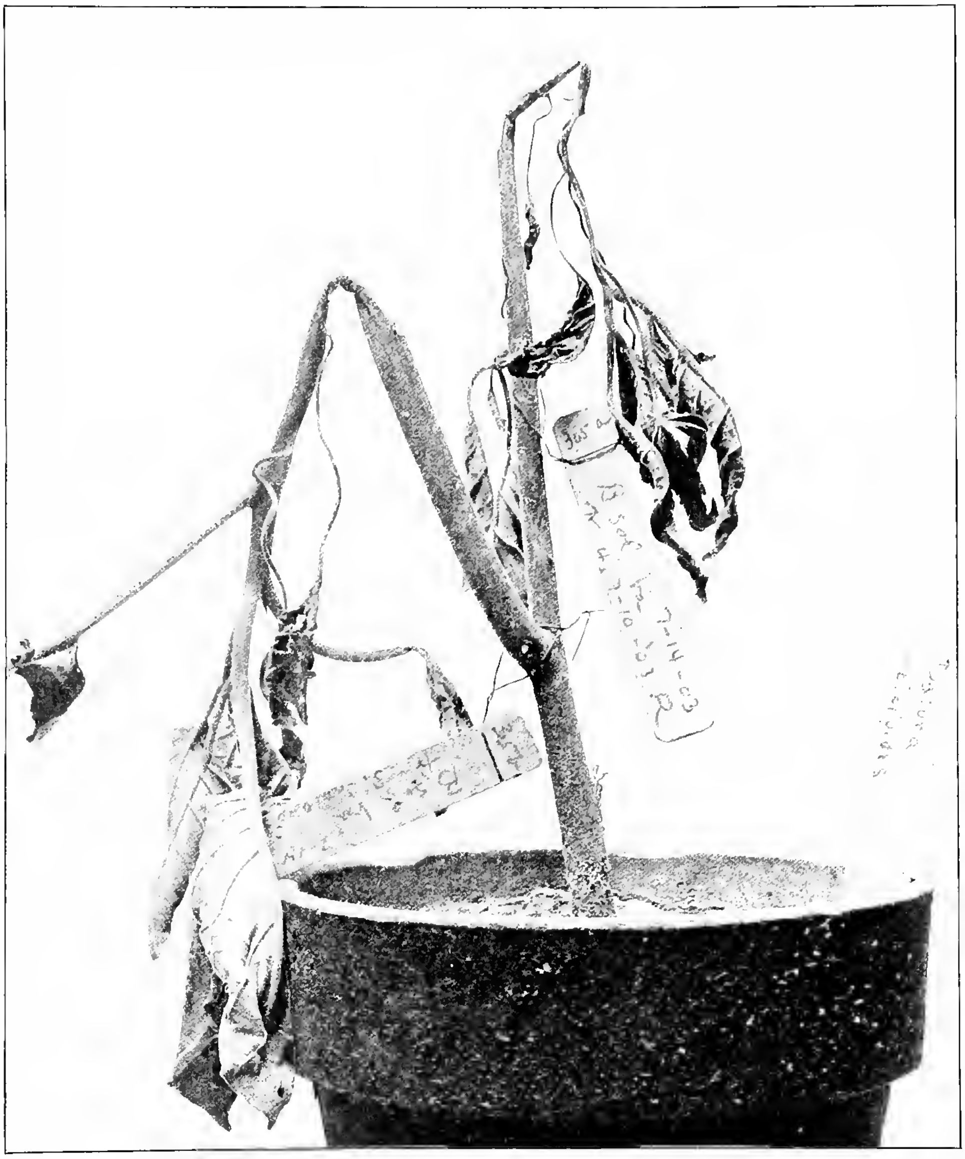


Fig. 10.*

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*Fig. 10.—*Datura metelloides,* inoculated by needle-pricks with *Bacterium solanacearum.* The same as plate 4, but six days later, *i. e.,* on July 28.

If all experimenters in plant pathology, even in recent years, had been careful to conform to these four rules of practice, the first three of which in essence were formulated by Robert Koch as long ago as 1882, some very deep chagrins might have been avoided.

Owing to insurmountable difficulties many animal pathologists, especially those who study human diseases, now frequently rely on the first two rules as sufficient, but, if possible, one should comply also with *c* and *d*. Plant pathologists are under no such limitations, and should conform to each one of the above-mentioned requirements, particularly if they desire their work to take high rank and to be generally accepted as conclusive. Material for plant-inoculation experiments is so cheap and easily procured that a writer who undertakes to describe a bacterial disease of plants has usually no good excuse for leaving any doubt whatever as to the pathogenic properties of the organism. There is also no excuse for limiting the inoculations to mixtures of bacteria or to crude material taken directly from the diseased plant, since every tyro in bacteriology now knows how to separate one organism from another in nutrient agar or gelatin by means of poured plates or Petri-dish cultures.

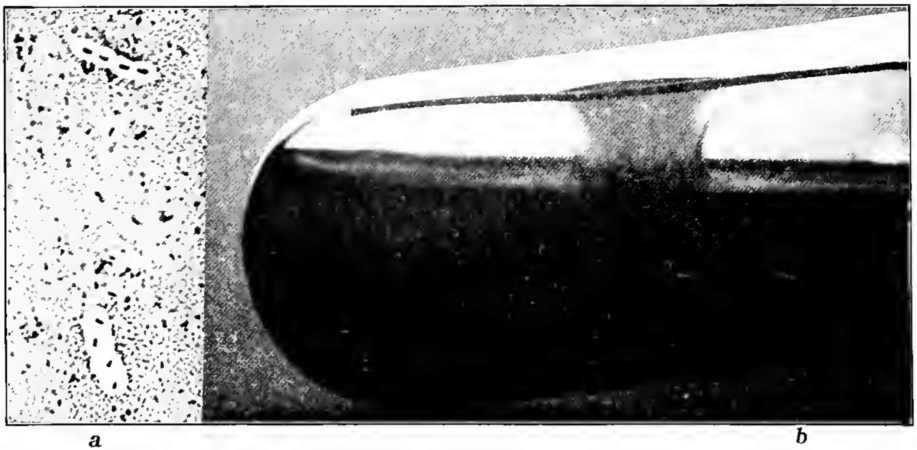

Fig. 11.*

MORPHOLOGY.

SIZE, SHAPE, ETC.

The smallest observed bacteria are only a small fraction of a micron in diameter. Migula states that the stained rods of *Ps. indigofera* (Voges) Mig. from colonies 36 hours old measured only 0.18 by 0.06 micron. *Bacillus denitrificans* (Amp. & Gar.) Mig. is also a very small rod—1.0 to 1.5 by 0.1 to 0.3 micron, according to Migula. *Micrococcus progrediens* Schröter is said to be only 0.15 micron in diameter. The organism of peri-pneumonia isolated by Nocard & Roux is more minute. It is probable also that still smaller organisms occur, *i. e.*, so small as to be invisible under the highest magnifications. In this way are interpreted the results obtained by animal pathologists in the foot-and-mouth disease and in some other diseases. Photographs with ultra violet light may in the end render some service here. The

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*Fig. 11.—*a*, Capsule of organism obtained from black spot of the plum. Bacteria grown in Uschinsky's solution and stained by Ribbert's method; *b*, ropy Uschinsky solution from which *a* was made.

largest bacteria are several thousand times as bulky as the smallest. Errera has described a spirillum the largest specimens of which measured 23 to 28 by 3 to 3.4 micra ('02, Errera, Bibliog., X), and Schaudinn has described a bacillus the largest forms of which are 24 to 80 by 3 to 6 micra ('02, Schaudinn, Bibliog., XI).

In shape the bacteria vary according to genera and species and sometimes within the limits of the species, from globose cells or very short straight rods, through curved forms or spirals, to filaments which are many times the diameter of the organism. To what extent does form vary under changed conditions? With the eye-piece micrometer make careful measurements of unstained organisms taken from the host-plant and from cultures of various ages and kinds. There is frequently considerable variability in the size of individuals of the same species. Is the breadth more constant than the length? Does the size or shape as observed in the plant differ from that observed on culture media? How does the living organism differ in size and general appearance from the dead, stained one?

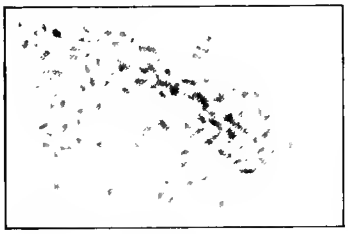

Fig. 12.*

CAPSULES.

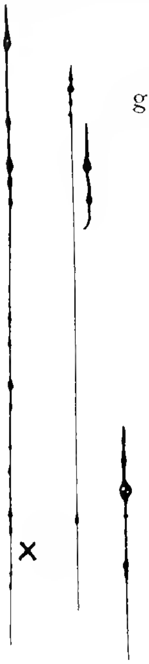

Fig. 13.†

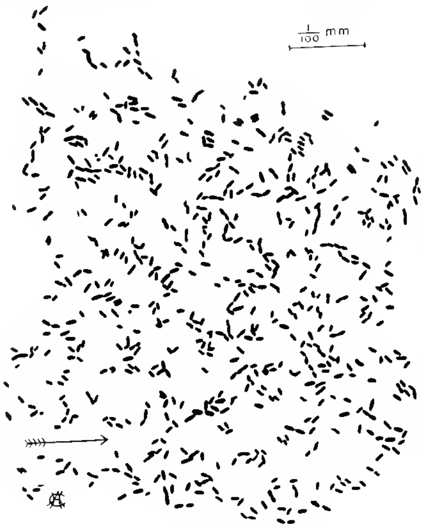

Fig. 14.‡

The presence of capsules may be suspected whenever a bacterial growth becomes viscid. They are often difficult to see because their index of refraction is so nearly that of the fluid in which they are usually examined. In examining unstained material the field should be illuminated with a narrow pencil of rays, and the effect of illumination with oblique light should be tried. Several methods of contrast staining are in use. By one method the capsule remains unstained or nearly so, while the central portion of the bacterium and the slime lying on the cover between the bacteria stain more or less deeply. By another method which has been specially commended by Dr. Welch ('92, Bibliog., XIII), the capsule is also stained, but remains distinctly paler than the body of the bacterium. They may also be counterstained, as in Muir's method or Moore's method. Well-defined capsules are shown in fig. 11*a*. This may be compared with fig. 12, in which the same organism is shown without capsules. Fig. 11*b* shows the extreme viscidity of a culture due to the formation and deliquescence of capsules. Fig. 13 shows the tenuous threads into which *Bacillus tracheiphilus* may be drawn as it oozes from the cut stems of cucurbits. Fig. 14 is a detail from the same more highly magnified, the viscid connecting substance being unstained.

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*Fig. 12.—A portion of the yellow ooze from the black spot of the plum, stained by ordinary methods. × 2,000.

†Fig. 13.—Cobwebby, sticky threads of *Bacillus tracheiphilus* drawn from the cut end of a muskmelon stem, arranged on a slide and stained with carbol-fuchsin. About three times natural size. Buzzards Bay, Mass., Oct. 8, 1903. Fig. 14 was drawn from the left-hand thread at the point marked X.

‡Fig. 14.—*Bacillus tracheiphilus* Erw. Sm. A portion of one of the threads shown in fig. 13. The arrow indicates the direction of the thread, which was extremely tenacious. The distance between the bacterial rods indicates very clearly the extreme viscosity of the unstained substance lying between them and holding them together. × 1,000.

FLAGELLA.

Ehrenberg was the first to describe flagella on bacteria (*Bacterium triloculare*, 1838). Nothing more was done until 1872, when Cohn discovered them on *Spirillum volutans*. In 1875 Dallinger & Drysdale saw and figured them on *Bacterium termo*. In 1875 Warming determined their existence on *Vibrio rugula* and *Spirillum undula*. In 1877 Koch demonstrated their existence on a number of species by the use of stains. In 1878 Dallinger, using unstained material, saw them many times on *Bacterium termo* and also on *Spirillum volutans*. After 1879 no one appears to have disputed their existence. In 1890 Messea proposed to divide the flagellate bacteria into four large groups, monotrichiate, lophotrichiate, amphitrichiate, and peritrichiate. In 1895 Fischer used the flagella as marks to distinguish subfamilies. In the previous year Migula used their number and mode of attachment as a means of distinguishing genera.

The staining of flagella has now become a regular part of laboratory work. Their number and position on the body wall should be determined, if possible, in case of each species studied. This is sometimes quite easy and at other times very difficult. It should also be determined whether the flagella are fugitive or persistent.

Flagella may be stained from young agar cultures. Bouillon cultures are to be avoided because of the intense ground stain. Some kinds may be stained readily from cultures grown for some days in a very dilute Uschinsky's solution—1 to 3 drops in 10 cc. of distilled water (fig. 15). The flagella of some bacteria are stained readily, those of others only with great difficulty. Many sorts seem inclined to throw off their flagella when transferred from agar to water. The cover-glasses must be *clean*. When cleaned ready for use seize with the forceps and pass them three times through the upper part of the Bunsen flame, with a considerable interval between each flaming, to avoid cracking. Use a minim quantity of the culture stirred in a big drop of water, or even in 2 to 10 cc. of water in a watch glass or test tube. Give the bacteria time to diffuse by waiting half an hour or more. Take the cover between the thumb and finger of the left hand, touch the end centimeter of a platinum needle to the water containing the bacteria, and sweep it deftly across the cover glass. In this way the fluid is spread in a very thin sheet over nearly the whole surface of the cover and is dry almost at once, with the bacteria well separated. If the fluid will not spread, then the cover is not clean and should be discarded. The bacterial sheet may be mordanted and stained at once, or first fixed by gentle heat. To avoid scorching, the cover should be held between thumb and finger when it is passed rapidly through the flame. Beginners usually burn the bacterial layer.

Smeary dark lines and other deceptive artefacts must not be mistaken for the flagella. The following methods have been tried by the writer and have given good results, but none can be depended upon always, and much time and patience are sometimes required to get good preparations of a refractory organism: Fischer's modification of Loeffler's stain; Moore's modification of Loeffler's stain; Van Ermengem's nitrate of silver method; Löwit's copper-sulphate fuchsin mordant, followed by Ehrlich's anilin-water gentian violet. (For other methods consult "Formulæ" and "Bibliography of General Literature," XII.)

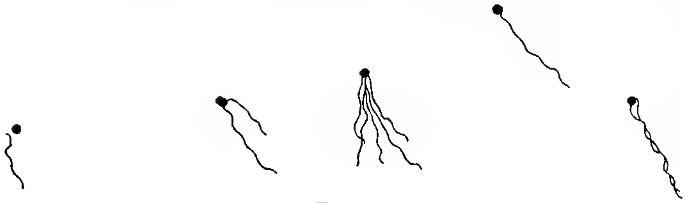

Fig. 15.*

In connection with flagella-staining a white porcelain tray, such as photographers use, will be found very convenient for washing, and also the double blow-bulb shown in fig. 17. This should be attached to a wash-bottle, such as that shown in fig. 16. This will deliver a small stream, very good for washing excess of mordant and stain from the covers. To furnish a steady stream the bulb has to be compressed only about once a minute. The flask used for this purpose should hold a liter.

SPORES—ENDOSPORES, ARTHROSPORES.

Do arthrospores really occur? If so, in what respect do they differ from the ordinary vegetative rods? Test spores for resistance to high temperatures in the water bath and to steam heat; study germination in hanging drops. Do the spores require a period of rest or refuse to germinate except in special media? The suspected existence of spores may be definitely settled by seeing the problematic bodies germinate. In the absence of such proof, considerable certainty may be reached by a combination of two methods: (1) the use of watery basic anilin stains, and (2) the use of moist heat. If at room temperatures the glistening bodies refuse to take the simple stains even on long exposure and at the same time are very resistant to steam heat or to hot water, *i. e.*, much more so than the ordinary vegetative rods, it may be assumed that they are spores. If, on the contrary, they are destroyed by temperatures only slightly above the recorded thermal death-point of the vegetative rods, it must not be assumed that they are spores, no matter how they behave toward

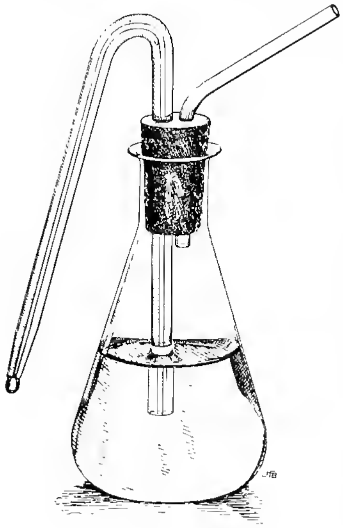

Fig. 16.†

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*Fig. 15.—Flagella of yellow organism plated from black spot of plum. Stained from culture grown in 10 cc. distilled water containing a few drops of Uschinsky's solution. × 1,000.

†Fig. 16.—Beyerinck's drop-bottle. The size and number of drops in a given time are regulated by sliding the bent tube through the cork. It is very convenient to have this flask on the microscope table. By a minim infection of the fluid it may also be arranged so that each drop shall deliver a single spore or bacterium for hanging-drop studies. About two-fifths natural size.

stains, unless they can be made to germinate. Many of the older identifications of spores are untrustworthy. Alfred Fischer has shown that many of these determinations rested on plasmolysis of the rods, *i. e.*, on misinterpretations. Omélianski reports finding an oval spore which stains readily with ordinary anilin stains. This occurs in a rather large bacillus accompanying his hydrogen cellulose ferment. Dannapple reports finding spores which are very sensitive to heat ('99, Bibliog., XXXIII). Usually only one endospore occurs in each cell, but Kern ('81, Bibliog., VIII), and Schaudinn ('02, Bibliog., XI) have found bacteria with two in each cell. Excellent directions for the study of spores are given in Part I of Migula's System der Bakterien (see especially the second paragraph on p. 209).

CELL-UNIONS—ZOOGLŒÆ, CHAINS, FILAMENTS.

In some media bacteria are much inclined to separate after division; in others they remain attached in various ways. The most common method of union is an irregular clumping, which in fluids gives rise to a fine or coarse flocculence. Such unions also occur on solid media and may be designated zooglœæ, or *pseudo-zooglœæ*, if one prefers to retain zooglœæ for the more intimately fused and compacter gelatinous unions. Sometimes the organisms remain attached end to end. Where the segmentation is distinct, such unions are designated chains. When very long and with obscure segmentation, they may be called filaments. Is there any true branching? What especial conditions of the culture medium favor the formation of zooglœæ, of chains, and of filaments? Many bacteria form zooglœæ, chains, or very long filaments under certain conditions, while under other conditions they remain as very short, straight rods. (Compare figs. 18 and 19.) As in case of involution forms unfavorable cultural conditions (thermal, nutrient, etc.) appear to have much to do with their appearance.

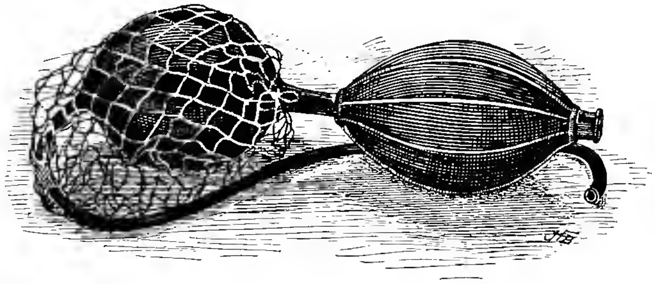

Fig. 17.*

The growth of bacteria may be studied in hanging drops of bouillon, etc. Hollow-ground slides (fig. 20) should be used for this purpose, rather than ring-cells, especially with high powers. Hill's hanging-block method is also serviceable ('02, Bibliog , XVII).

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*Fig. 17.—Double blow-bulb for attachment to drop-bottle shown in fig. 16. By use of this device one obtains with a minimum of pumping a constant small stream of water very suitable for washing stained covers, etc. Made by Emil Greiner. It is best used with a larger flask than that shown in fig. 16. Bulbs which have been long in stock should not be purchased, as the rubber deteriorates rapidly.

INVOLUTION FORMS.

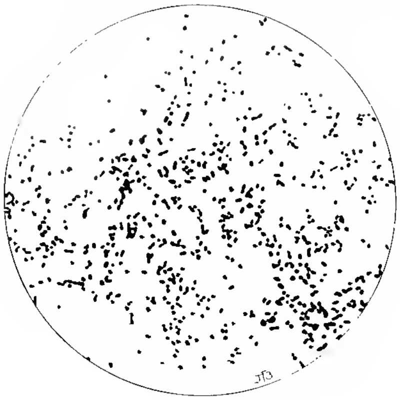

Fig. 18.*

Under this name we designate swollen and distorted forms common in old cultures (fig. 21). Under what conditions do they occur? Are they living or dead? Isolate in hanging drops of bouillon and determine whether they are stages in development or only degeneration forms. Are Y-shaped or branched forms such as occur in old cultures of *B. tuberculosis* Koch, and in the root-tubercles of clover (fig. 22) to be considered as involution forms? Are such organisms fungi or bacteria? Branching forms have been detected by many observers. (Consult numerous citations in the Bibliography of General Literature, X). The most recent paper is by Albert Maassen (Arb. a. d. Kais. Gesundh., Bd. XXI, H. 3, 1904, p. 377, 6 pl.). He found chloride of lithium specially advantageous for provoking these growths, which are regarded as teratological. He obtained them in 24 hours.

GENERAL COMMENT.

Great care should be paid to the minute morphology of each organism, not only in the host-plant but also in a variety of cultures, old and young, so that a body of knowledge more exact than we now possess shall be gradually accumulated for differential and systematic purposes. Careful drawings and photographs should be made. The Abbe camera is a great help in making drawings (fig. 121). For such study the Zeiss apochromatic lenses and compensating oculars can not be recommended too highly, particularly the 16 mm., with the 12 and 18 compensating oculars for studying the margins of colonies, and the 2 mm. 1.30 n. ap., with the 8 and 12 compensating oculars for the more detailed study of the individual rods. The writer has also made much use of the Zeiss 3 mm. 1.40 n. ap. apochromatic objective. The Zeiss screw, or filar, micrometer combined with a No. 12 compensating ocular (fig. 23) will be found very useful. For photographic purposes the projection oculars or the 4 or 6 compensating oculars may be used. Robert Koch was entirely correct in saying: "A general use of photography in microscopic works would certainly have prevented a great number of unripe publications."

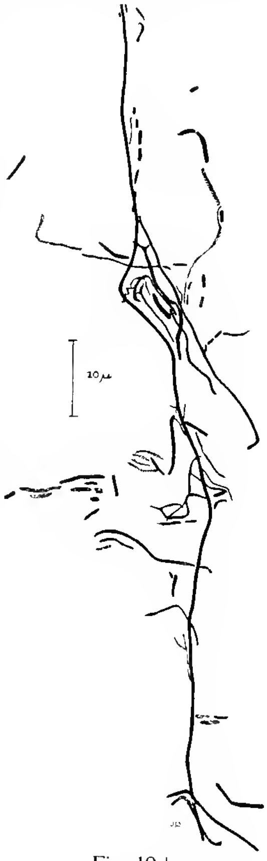

Fig. 19.†

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*Fig. 18.—*Bacterium campestre.* Cover-glass (smear) preparation from the vessels of a cabbage plant received from Racine, Wis., Sept. 19, 1896. Stained with carbol-fuchsin. Drawn from a photomicrograph. × 1,000 circa.

†Fig. 19.—*Bacterium campestre* from an old culture on 23 per cent grape-sugar agar, showing long filaments. Cover stained 1 hour and 20 minutes in gentian violet (1 part saturated alcoholic solution plus 1 part water). Many of the rods stained feebly. Tube inoculated June 30, 1898. Cover prepared Aug. 8. Drawn directly from the slide. × 1,000.

Good photomicrographs should be secured if possible. Koch's first photomicrographs were of various enlargements. He afterwards recommended × 1,000 as the standard magnification, but × 1,500 and × 2,000 are also convenient sizes and occasionally × 500 is better than × 1,000. Most important is it that the exact magnification should always be indicated. The Zeiss apochromatic objectives are much better for photographic work than the achromatic ones. For very small magnifications the writer has found the old Zeiss 35 mm. and 70 mm. very useful. For the same purpose the newer Zeiss planars, series Ia Nos. 1–5 (fig. 122) are admirable. These have sharp definition and a very flat field, but not much depth of focus. With them objects several centimeters in diameter may be satisfactorily photographed with magnifications from 2 or 3 diameters to 50 or more. The writer obtains as sharp a focus as possible with wide-open diaphragm and then stops down about two-thirds.

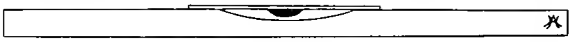

Fig. 20.*

One of the best simple photomicrographic outfits is the Zeiss upright camera (fig. 24). All apparatus is to be rejected which requires the microscope to rest on the same platform as the camera. It should rest on the table independent of the camera, unless a weak light is used and the exposures are very long, in which case a slight jarring is of no great consequence. Direct sunlight is the best light, but the light of the open sky may be used (with full open diaphragm) if one is willing to make 5 to 20 minute exposures. Electric light is often used by those who live in cloudy regions or who occupy rooms not exposed to the sun, but the writer has had no experience with it. Very good pictures also may be made by gaslight if the Welsbach burner is used. Ordinary lamp light (kerosene) is too yellow and not sufficiently intense. Photographs can be made with a kerosene light, but the time and trouble involved make it scarcely worth while to consider this source of light. The writer has obtained the best results by using direct sunlight and slow isochromatic plates behind Zettnow's light filter. Of course, with upright cameras a dry light-filter must be used, such as the yellow one devised by Carbutt or by Ives. In using a horizontal apparatus, such as that shown in plate 5, the *sine qua non* is to get it properly leveled up and to keep it so.

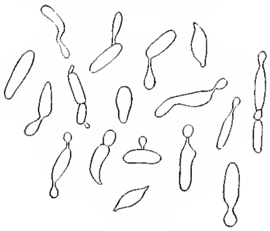

Fig. 21.†

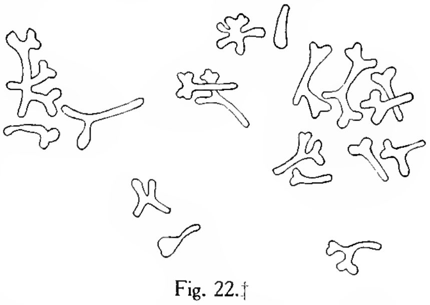

Fig. 22.‡

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*Fig. 20.—Hollow-ground slide with cover-glass bearing hanging drop for examination under the microscope.

†Fig. 21.—Involution forms of *Bacillus tracheiphilus* from extremely ropy potato broth. Drawn free hand, × 1,000 circa. Many as large as 8 by 2 micra and others larger. Nov., 1894.

‡Fig. 22.—Y-*shaped* (dichotomously branched) bodies from the root-tubercles of clover (Trifolium). From a photomicrograph by the author, made from a slide furnished by Dr. Geo. T. Moore. × 1,500.

For the inspection of colonies and of subcultures in tubes the best hand-lens known to the writer is the Zeiss aplanat magnifying six times (fig. 25). That magnifying 10 times is also very useful, but will not reach to the center of an ordinary test tube. Those in apple-tree wood cases are in some respects more convenient than those provided with metallic swing covers (fig. 26).

The best general work to consult on the morphology of the bacteria is undoubtedly Migula's System (see Bibliog., III).

Physiology.

In the description of bacteria we are compelled to make large use of physiological peculiarities, owing to their very simple and monotonous morphology. Within the limits of the genera now recognized the form differences are so very slight that many bacteria, *e. g.*, *Bacillus coli*, *B. cloacæ*, *B. suipestifer*, *B. typhosus*, *B. amylovorus*, etc., are indistinguishable under the microscope. In mixed cultures, or stained preparations, no one could distinguish one from the other with any certainty, and in pure cultures of unknown origin certain identification by means of the microscope would be equally impossible. Nevertheless, these same forms are so widely different in their behavior in culture media, in their pathogenic properties, in their relation to heat, air, antiseptics, etc., that we are certainly warranted in regarding them as distinct species, using the word "species" in its common acceptation. These well-ascertained facts should not, however, lead one to neglect slight differences of form, even when they can be expressed only in fractions of a micron. On

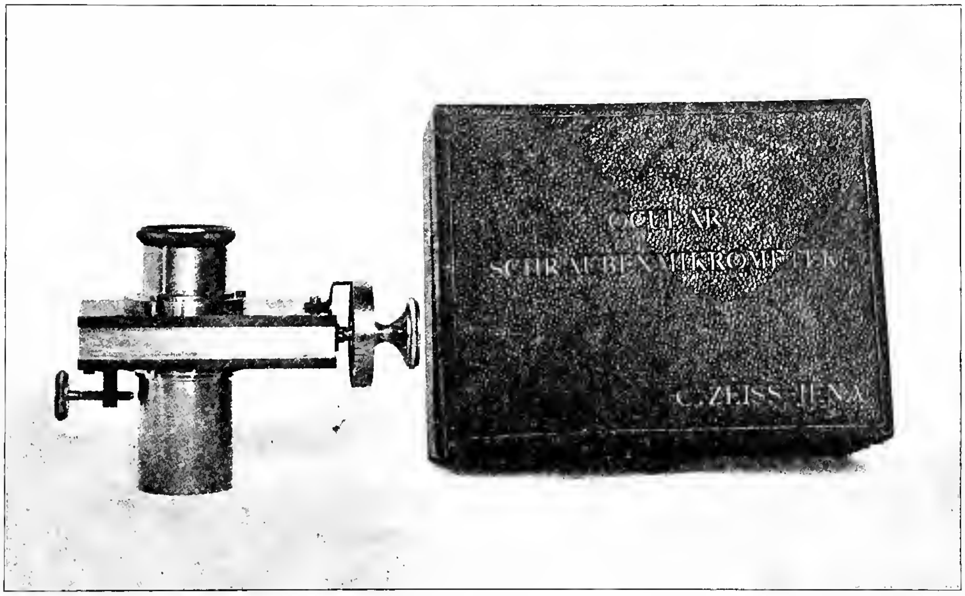

Fig. 23.*

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*Fig. 23.—Zeiss compensating ocular No. 12 with screw-filar micrometer.

the contrary, as much as possible should be made out of morphology, particularly that of the living organism, and in this connection the recent efforts of Migula and Fischer are especially deserving of commendation.

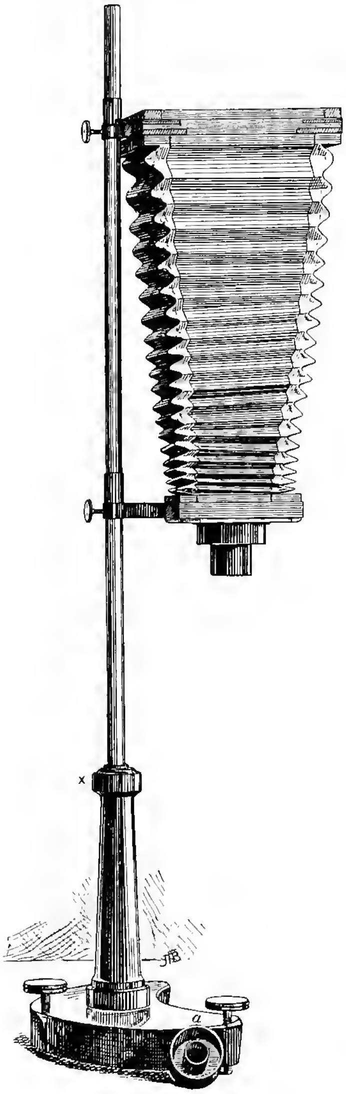

Fig. 24.*

MOTILITY.

If motile, determine kind of motion and rapidity (margin of small hanging drops on thin covers suspended over hollow-ground slides).† The cover may be prevented from sliding by touching one edge with a *very little* vaseline or cedar oil; if too much is used it runs under, mixes with the hanging drop, and spoils the mount, and possibly in the end the objective is ruined, if the student continues to search for a clear field. The beginner is very apt to mistake Brownian movement for self-motility. It sometimes requires very careful observation to be quite certain. Rods which appear to be motionless will sometimes be seen to dart away quite suddenly if watched. In some species young cultures are much more apt to be motile than old ones; in others motility appears to be an almost constant characteristic. The movements of bacteria are sometimes quite characteristic for particular sorts. They may be slow or rapid tumbling motions centering in the shorter axis, or straight or sinuous slow or rapid darting movements in the direction of the longer axis, with rotation on this axis. The media of Hiss ('97, Bibliog., XVI) and of Stoddart ('97, Bibliog., XVI) are sometimes useful for distinguishing macroscopically between motile and non-motile forms. The former spread as a thin layer over the whole surface, the latter pile up in restricted areas around the points of inoculation. The student should not remain content with merely determining motility, but when this has been settled he should turn his attention to staining the organs of motion.

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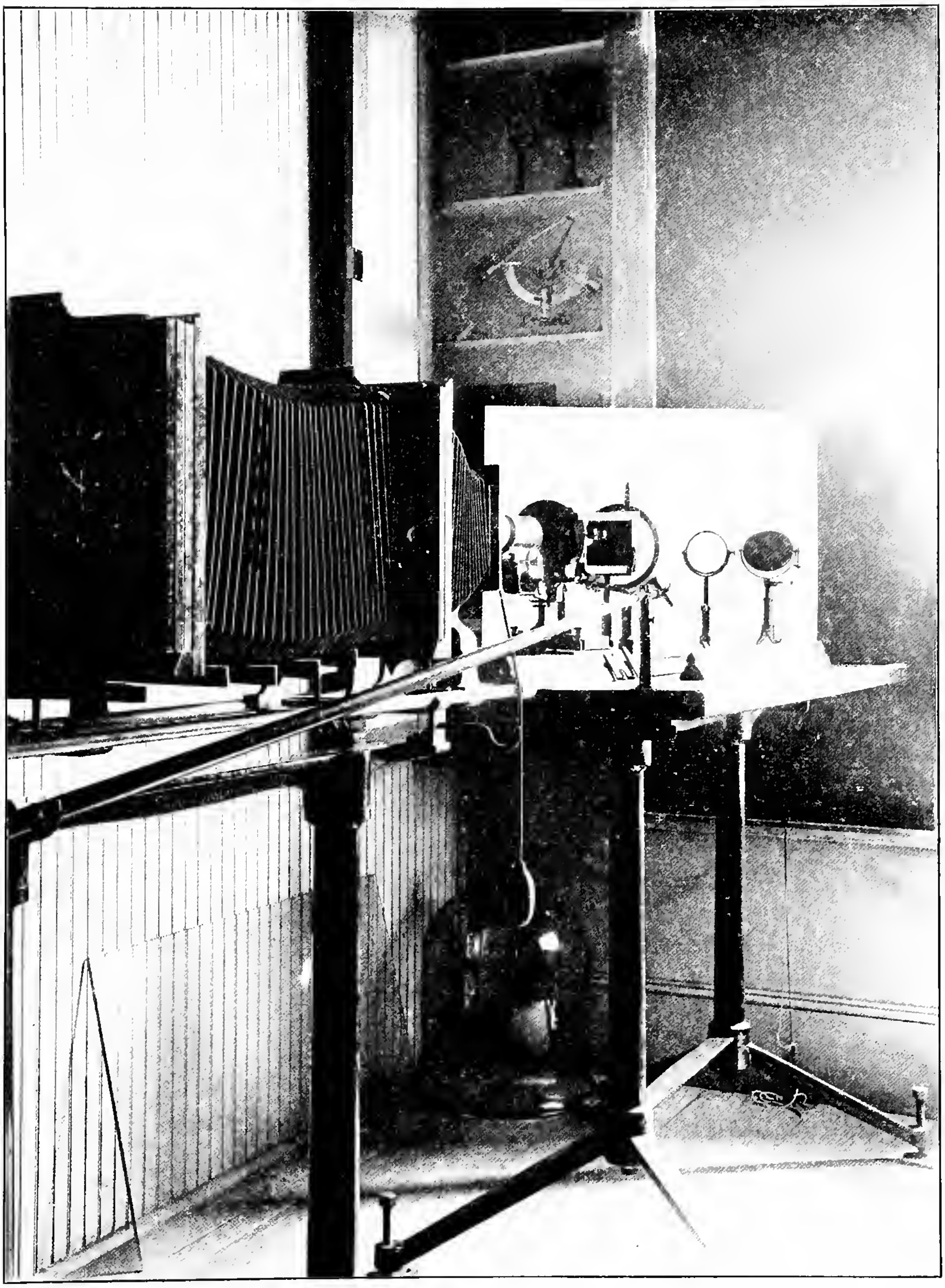
*Fig. 24.—Upright Zeiss camera for photomicrographic work. The cup (*a*) slips over the end of the microscope and forms a light-tight connection with the bellows without touching it. The microscope rests on the table independent of the camera. The stout rod turns freely in the socket X and is locked in place by a set-screw on the side opposite the observer. The height is about 45 inches.

†Lehmann and Fried (Arch. f. Hyg., Bd. XLVI, 1903, p. 311) found the swiftest movement of bacteria to be 1 mm. in 22 seconds; the slowest 1 mm. in 222 seconds; average: cholera, 1 mm. in 34¼ seconds; typhoid, 1 mm. in 56 seconds; *B. vulgare*, 1 mm. in 73 seconds; *B. subtilis*, 1 mm. in 40 seconds; *B. megaterium*, 1 mm. in 2 minutes 11 seconds.



Large horizontal Zeiss photomicrographic outfit ready for use,

except that when photographing the curtain is raised and the mirror is placed farther away, i. e., out of the south window on the triangular extension shown on the front table at the right. In the newer forms each table top may be raised or lowered at will. There is also a device for raising or lowering the plate on which the microscope rests.

GROWTH.

The manner of growth and rapidity of growth at given temperatures in hanging drops and also on the margin of young colonies on plates of nutrient gelatin and agar of varying density should be determined. Frequently characteristic and interesting arrangements of the rods forming the surface layers of the colony, especially when it is young, may be discovered by means of a direct inspection of the colonies under low powers of the microscope or by means of cover-glass impressions. Covers are carefully placed on the colony, removed, dried, flamed, and stained. There are also often curious arrangements of the deeper layers of the surface colony. In direct examination the colonies should be viewed by reflected as well as by transmitted light. Drawings or photographs of surface colonies should be made under low or medium magnifications. By a little practice using Lister's dilution method ('78, Bibliog., XVII), hanging-drops containing a single bacterium for study under the microscope may be obtained with Beyerinck's capillary drop-flask ('91, Bibliog., XVII).

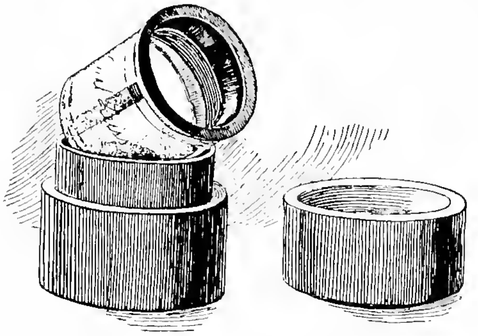


Fig. 25.*

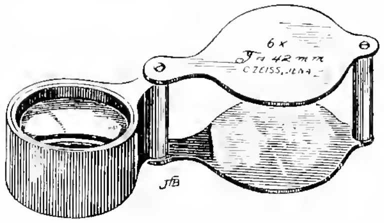


Fig. 26.†

CHEMOTROPISM.

On the general subject of chemotropism, see papers by Pfeffer, Miyoshi, Jennings, Buller, Rothert, etc. Jennings maintains that contact irritation inducing motor reflex is responsible for movements which were formerly attributed to chemical stimulus. Consult Jennings, "Contributions to the study of the behavior of lower organisms," Carnegie Institution of Washington, 1904, and especially Jennings and Crosby, "The manner in which bacteria react to stimuli, especially to chemical stimuli," Am. Jour. Physiol., Vol. VI., pp. 31-37, and Jour. Roy. Mic. Soc., 1902, p. 88. *Spirillum volutans* was used in the tests.

REACTION TO STAINS.

Proper staining is a very important part of the study of bacteria. Its foundation principle is the fact that the bacteria, in a living vegetative condition, all show a great affinity for the basic anilin dyes. Spores ordinarily show no such affinity, but may be made to take up stains by acting on them with strong acids or alkalis, or by heating them very hot. Flagella also show no affinity for stains until acted on by severe reagents, when they may be stained in mordanted solutions or in dyes which have been preceded by a mordant. The outer wall of the bacterium generally reacts to stains in the same way as the flagella, *i. e.*, it usually remains unstained.

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*Fig. 25.—Hand lens suitable for examining bacterial cultures. Zeiss aplanat magnifying six times. Three-fourths natural size.

†Fig. 26.—Zeiss swing-cover aplanat magnifying six times. This is now sent out in a neat little chamois-skin purse. About two-thirds natural size.

Staining media may be roughly divided into four groups: (*a*) Simple stains dissolved in water, *e. g.*, fuchsin (basic), gentian violet, methylene blue; (*b*) alcoholic solutions and various complex stains, *e. g.*, saturated alcoholic solutions of anilin dyes, alcohol-iodine, iodine potassium iodide, Russow's cellulose test, Ziehl's carbol-fuchsin, Loeffler's alkaline methylene blue, Ehrlich's anilin-water gentian violet, Gabbett's stain, Gram's method, Delafield's hæmatoxylin, Ehrlich's acid hæmatoxylin, Heidenhain's iron-hæmatoxylin, Fleming's triple stain; (*c*) flagella and capsule stains, *e. g.*, Loeffler's stain, Moore's modification, Fischer's modification, Bunge's stain, Löwit's stain, van Ermengem's nitrate of silver method, Zettnow's gold method, etc.; (*d*) stains for spores, *e. g.*, prolonged exposure to simple stains dissolved in water (which should have little effect), steaming carbol-fuchsin with methylene blue for contrast, etc. (see "Formulæ" and Bibliography of General Literature under "Flagella," "Spores," etc., for various observations on staining).

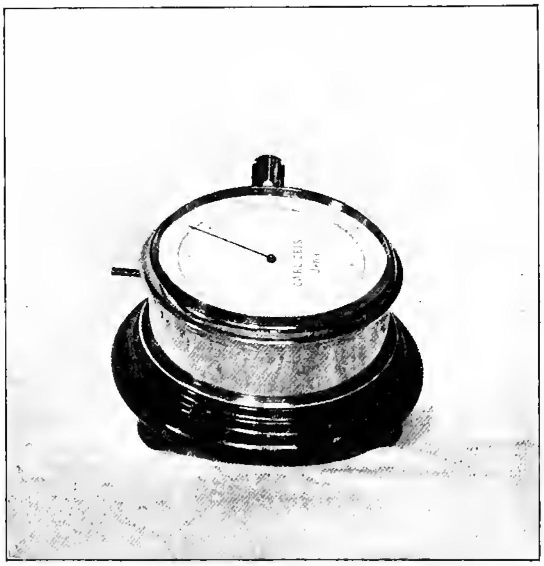

Fig. 27.*

Grübler's stains are preferred. Cover-glasses should be clean and free from fat, traces of which are easily removed in a Bunsen flame. A little experience is necessary in flaming thin covers in order not to crack them. They may be passed through the flame three times, waiting a moment or two after each pass for them to cool. The student should see that the water used in making the cover-glass preparations or the stains does not itself contain bacteria. It is usually wise first to dry a drop of the water on the cover and stain without addition of the bacteria. Eternal vigilance is the price of trustworthy results. It is best to make all mounts on cover-glasses of a known and uniform thickness (0.15 mm.). Many a good preparation has been spoiled for examination with lenses of a short-working distance by mounting under a thick cover-glass, and sometimes the lens itself has been ruined in the attempt to focus. The thickness of covers often varies greatly from the statements of dealers, and they should not be accepted until tested with a reliable cover-glass measurer (fig. 27).

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*Fig. 27.—Zeiss cover-glass measurer. The cover in place shows a registered thickness of 0.18 mm. Fractions of an inch are also registered on this instrument.

To determine whether bacteria are properly stained examine with the diaphragm of the condenser wide open. If they can not be seen distinctly with this flood of light they are not well stained. The bacteria should be well separated on the cover and deeply stained, while the background should be very free from stain.

Dr. Weigert seems to have been the first to use anilin stains for the demonstration of bacteria in tissues. This was about 1875. Since that time staining in tissues has been worked up carefully for bacteria causing animal diseases, but very little is known respecting best methods of staining bacteria in vegetable tissues. The difficulty lies in the fact that the tissues of the higher plants often take the basic anilin stains as readily as the bacteria and retain them even more tenaciously. Special remarks may be looked for under particular diseases.

CULTURE MEDIA.

NUTRIENT GELATIN.

(*a*) *Plate Cultures.*—Colonies, young and old, buried and superficial, crowded and wide apart, should be examined for color, translucency or opaqueness, shape, thickness of the surface growth, and character of the margin. They should also be studied under low powers of the compound microscope for lobes, branches, granulations, wrinkles, flecks, concentric rings, radial filaments, arrangement of the dividing rods on the margin of the colony, iridescence, etc. The microscopic appearance of the surface colony during the first 48 hours is often different from that later on. The rapidity of growth should be compared with that of some common and easily accessible organism, *e.g.*, *Bacillus coli*, *B. amylovorus*, *Bacterium campestre.* The comparative rate of growth of buried and surface colonies should also be carefully noted. How is the appearance of the colony changed by increasing the amount of gelatin, or varying the brand of gelatin? Are the surface colonies viscid, or can they be lifted bodily in one mass from the substratum?

(*b*) *Stabs.*—The nature of the surface growths and of the deeper growths should be carefully examined. Is there any marked tendency of the latter to grow downward or outward into the body of the gelatin, either in distinct masses or as a diffused cloudiness? Observe effect, if any, on growth when the gelatin is acid or only feebly (litmus) alkaline. If liquefaction of the gelatin occurs, note its rapidity and whether it is mostly restricted to the surface or is equally rapid along the line of the stab in the depths; note also whether the liquefied gelatin is clear or cloudy in tubes which have not been shaken, and whether a pellicle has formed on its surface. Liquefaction may be very rapid (taking place within a few hours), may occur after three or four days, may be long-delayed and feeble (only visible after some weeks), or may not occur at all. It is the cases of feeble and long-delayed liquefaction which lead to contradictory statements on the part of different observers, and consequently cultures should remain under observation for a considerable time and on a variety of gelatins. Various substances interfere with liquefaction. Determine whether liquefaction can be prevented by the addition of grape-sugar or cane-sugar (10 per cent). Look for gas-bubbles, for crystals, for any fluorescence or staining of the medium (green, brown). Inasmuch as the growth of some bacterial plant parasites is restrained by some nutrient gelatins which are neutral or only feebly alkaline to litmus, it is advisable to add to a part of the stock more caustic soda than is commonly used, *i. e.*, enough to render it neutral to phenolphthalein (strongly alkaline to neutral litmus), especially if gelatin is selected as the first medium for isolation experiments; otherwise perplexing failures may result.

(*c*) *Streaks.*—Record the character of the streak, whether wet or dry, smooth, wrinkled, or rough, thin or piled up, margin well defined or indistinct. Note also whether the surface is ever iridescent, whether growths are sent down from the under surface into the substratum, whether the streak spreads rapidly and widely over the surface or very slowly. The surface behavior depends to some extent on the motility of the organism, on the amount of water in the surface layers, *i. e.*, whether the slants are fresh or old, and on the amount of gelatin in the medium, which in temperate climates should usually be 10 per cent, but may be 15 or even 20 per cent. By minimizing heat in preparation and by increasing the quantity of gelatin to 20 or 30 per cent a medium may be obtained which will remain solid at 30° C. Growth is less satisfactory, however, on such a dense medium, or at least was in the few tests made by the writer. Chester has applied the ordinary botanical terminology to the varying margins of colonies, etc., and has published some useful figures ('01, Bibliog., III).

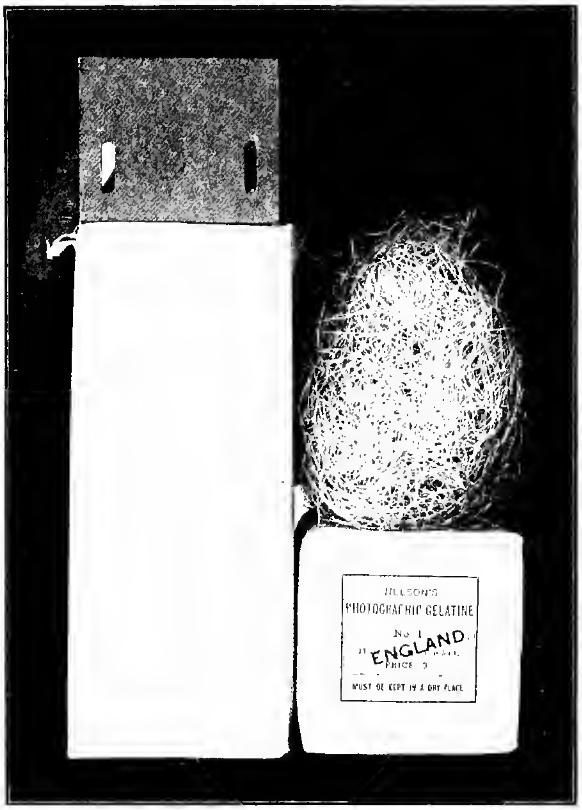

Fig. 28.*

No substance used in the bacteriological laboratory is so uncertain and variable in its composition as gelatin. The gelatin from different factories varies greatly and hardly any two batches from the same factory are alike. One glue chemist has defined gelatin as "80 per cent glue, 10 per cent dirt, and 10 per cent doubt." It varies greatly in its melting point and power of setting, and in amount of peptones and albumoses it may contain, which is sometimes large. It always contains calcium salts and phosphates, which are often antiseptic, and the nature of which varies according as hydrochloric or sulphurous acid has been used in its manufacture. Formaldehyde is sometimes added to it, we are told; and occasionally agar also, it is said, is added to certain table gelatins to increase their body. Gelatin also contains a variety of decomposition products due to the growth in it of various fungi and bacteria while it is in the vats or in the drying-house. If there is any delay in the drying it is spotted all over with molds and bacteria. It also contains some wax or grease, used to anoint the surface on which it is spread to dry, and this wax or grease is probably also a variable substance. Gelatins also polarize, it is said, in many different ways. An absolutely pure gelatin of uniform character for bacteriological purposes is not to be had. That which perhaps comes the nearest to it and which is here recommended is Nelson's gelatin, made in London and well known to the makers of photographic dry-plates, who use it in large quantities. It comes in two grades, a hard and a soft, and costs about $1.25 per pound. No. 1, that which I like best, comes in shreds resembling "excelsior" used for packing (fig. 28). No. 3, which comes in long, broad strips, contains much cell detritus, etc., and filters with difficulty. Other expensive gelatins, said to be of quite uniform quality, are Lichtdruck gelatin, made by Carl Creutz, Michelstadt, in Hesse, and Geneva Red Cross gelatin made by Winterthur, in Switzerland, under direction of Dr. Eder, of the Imperial Institute of Vienna (Cockayne).

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*Fig. 28.—Nelson's photographic gelatin No. 1. Recommended for bacteriological use.

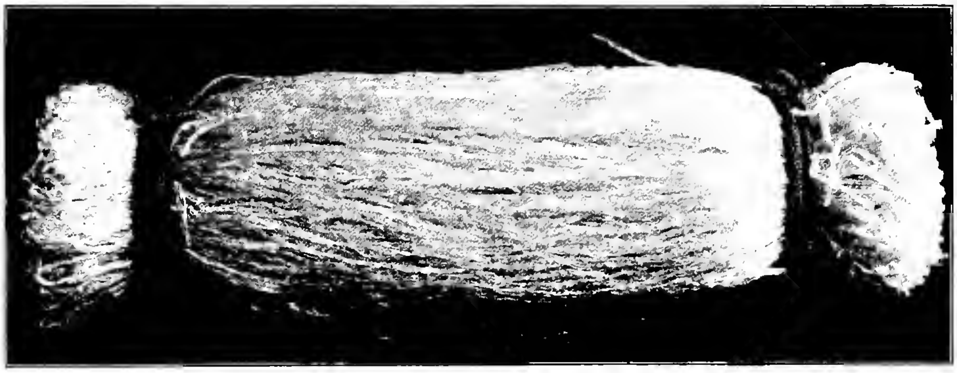

Fig. 29.*

NUTRIENT AGAR.

Agar, or agar-agar, as it is usually called, from a Malay word meaning "vegetable," is a manufactured product obtained from various sea-weeds growing in Chinese and Japanese waters. Various species are used as food and the trade is considerable. It usually comes into the hands of the bacteriologist as long, slender, yellowish-white strips (fig. 29) or as blocks (fig. 30), or more especially in recent years, in the form of a gray-white fine powder of European manufacture (fig. 33). It is reputed to be the product of species of Gelidium (figs. 31 and 32).

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*FIG. 29.—The kind of agar-agar usually employed in bacteriological work. This is a manufactured product known to the Japanese as slender "Kanten." The figure represents first quality "Kanten," in unbroken package. (Courtesy of Dr. Hugh M. Smith, Deputy Commissioner of the United States Bureau of Fisheries, who brought the package with him from Japan.)

Of the Japanese algæ in this group the following, according to Rein (pp. 81-82), deserve special mention:

(1.) *Chondrus punctatus* Sur.

(2.) *Gigartina tenella* Harvey; Jap. Ogo.

(3.) *G. intermedia* Sur.

(4.) *Gloiopeltis tenax* Kg. (*Sphaerococcus tenax* Ag.)

(5.) *Gl. capillaris* Sur.; Jap. Shiraga-nori.

(6.) *Gl. coliformis* Harv.; Jap. Kek'Kai.

(7) *Gl. intricata* Sur.; Jap. Fu-nori.

(8.) *Gelidium corneum* Lamouroux; Jap. Tokoroten-gusa.

(9.) *G. Amansii* Lamour.

(10.) *G. cartilagineum* Gail.

(11.) *G. rigidum* Grev.; Jap. Tosaka-nori, *i. e.*, cockscomb algæ.

(12.) *Sphaerococcus confervoides* A.; Jap. Shiramo.

(13.) *Gymnogongrus flabelliformis* Harv.; Jap. Home-nori.

(14.) *G. japonicus,* Sur.; Jap. Tsuno-mata.

(15.) *Kallymenia dentata;* Jap. Tosaka-nori.

(16.) *Porphyra vulgaris* Ag.; Jap. Asakusa-nori.

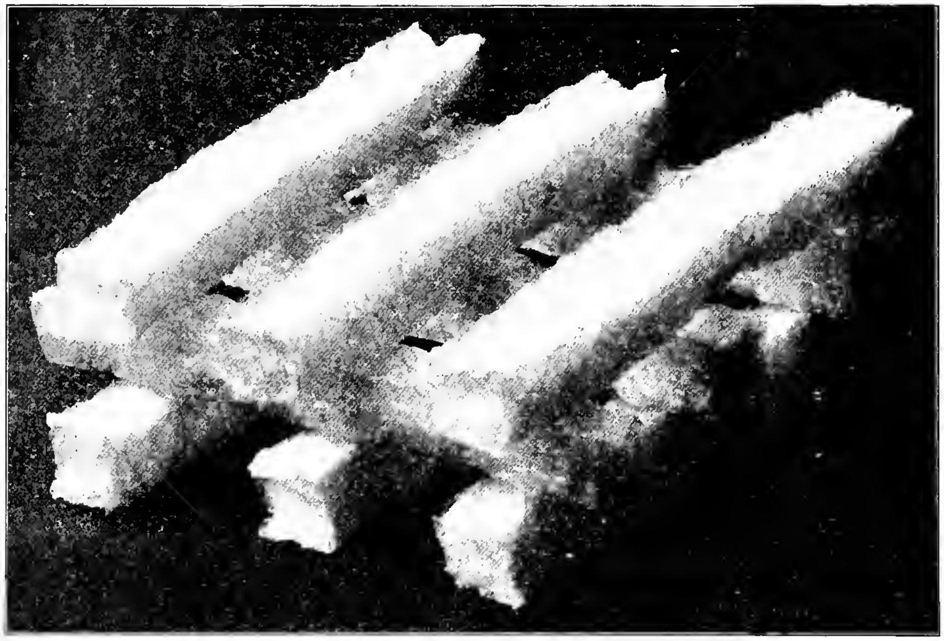

Fig. 30.*

Agar-agar is a neutral or nearly neutral substance which is converted by boiling with water into a stiff jelly that hardens in 1 per cent solution at 39° to 40° C., and is not easily liquefied either by the growth of organisms† or by heat less than that of boiling water. It is a kind of vegetable glue forming a good matrix for various nutrient substances. A chemical analysis by Karten (Descript. Cat. Int. Health, Exhib., London, 1884) gave the following proximate composition: 11.71 per, cent nitrogenous matter (albumen [?]), 62.05 per cent non-nitrogenous matter (evidently glue, the pararabin of Reichardt), 3.44 per cent ashes, and 22.80 per cent water.

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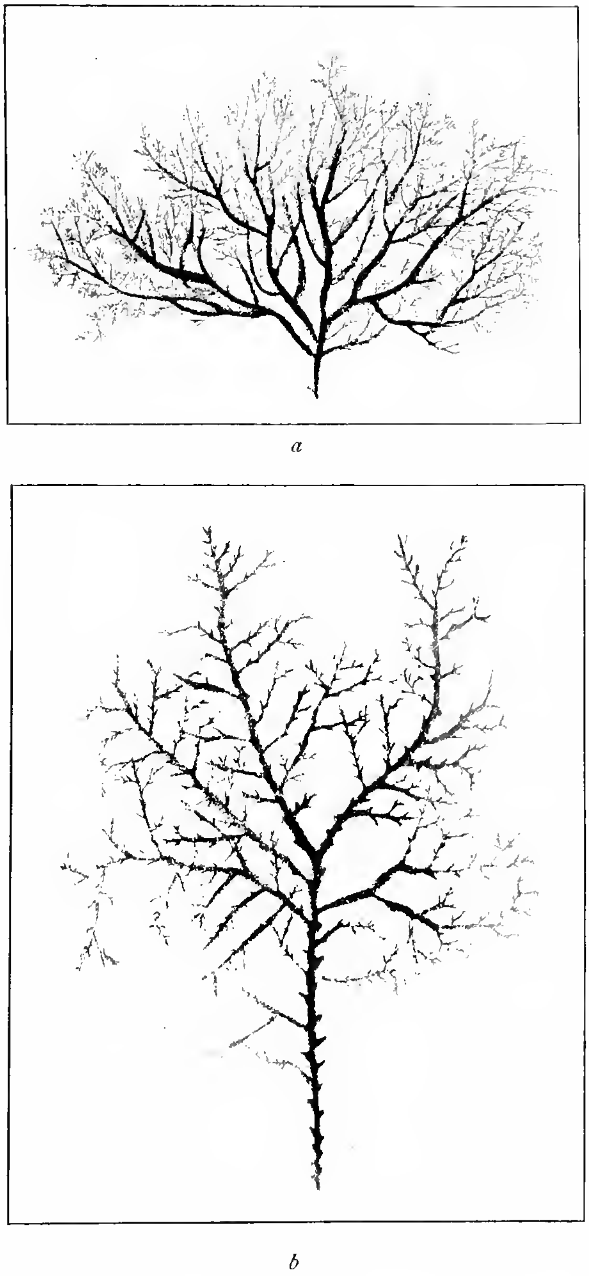
*Fig. 30.—Another form of agar-agar known to the Japanese as square "Kanten." The bulk of this goes to Holland, where it is used for clarifying schnapps. Courtesy of Dr. Hugh M. Smith. The actual size of these sticks is about 10¼ by 2½ by 1¼ inches.

†Metcalf has described a bacillus which slowly softens it, and the writer has observed similar phenomena.

For a full account of Japanese methods of making agar-agar consult a paper entitled "The Seaweed Industries of Japan," by Dr. Hugh M. Smith, in the Bulletin of the United States Bureau of Fisheries for 1904.



a

b

Fig. 31.*

In addition to beef bouillon, or in place of it, various substances, organic and inorganic, may be added to the agar with advantage. The writer makes much use of *litmus-lactose agar*, which is made out of ordinary nutrient agar by adding 1 per cent milk-sugar and enough pure litmus water to give a purple-red color. *Glycerin-agar*, *maltose-agar*, etc., may be made up with any amount of the substance desired, generally 1 or 2 per cent.

Formerly it was difficult to filter agar perfectly clear and it was therefore used less than gelatin, but in recent years it has been discovered that this difficulty may be overcome if the agar is first brought into complete solution by prolonged boiling or by a short boiling at a temperature somewhat above 100° C., *e. g.*, 110° C.

The writer formerly obtained filtered clear agar by soaking the snipped agar in 5 per cent acetic-acid water for some hours, after which a thin cloth was tied over the mouth of the beaker securely, and tap water allowed to run into it for an hour or more *i. e.*, until all trace of acid was removed. The softened agar was then put into the bouillon, boiled for two hours, and finally filtered through S. & S. filter

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*Fig. 31.—Red sea-weeds from which agar-agar is manufactured. *a, Gelidium corneum* Lam., one-third natural size; *b, Gelidium subcostatum* Lam., one-half natural size. From a colored Japanese chart showing "The principal aquatic plants of Japan," supposed to be an official publication. Original in the library of the United States Fish Commission.

paper,* using a hot-water funnel. Later he followed Schutz's method ('92, Bibliog., XVI), which is a very good one. This consists in cutting the agar into small bits and first heating it very hot in a beaker or enameled-iron dish in a minimum quantity of water or beef-bouillon over a hot Bunsen flame with constant and rapid stirring and

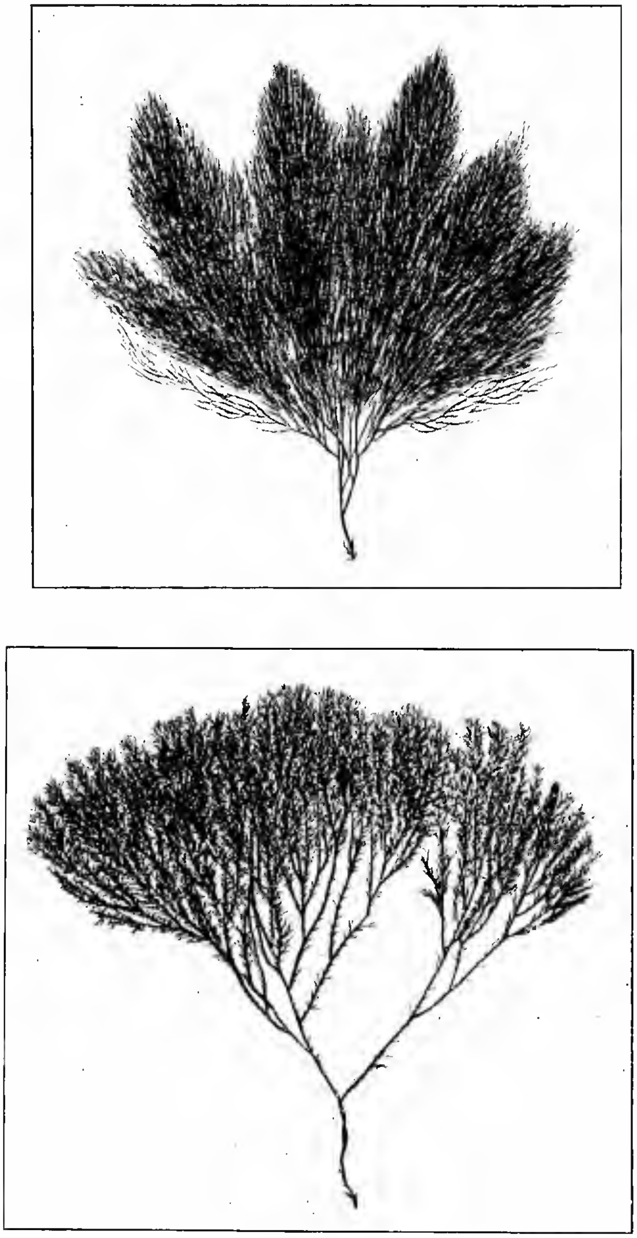

Fig. 32.†

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*The folded filter papers are the most convenient (fig. 34). These filter papers give the starch reaction (blue) with iodine, and reduce Fehling's solution on being boiled in it.

†Fig. 32.—Unnamed species of red sea-weeds (*Gelidium*) furnishing agar-agar. From a Japanese chart showing "The principal aquatic plants of Japan," supposed to be an official publication. One-half natural size. Original in library of United States Fish Commission.

occasional additions of small quantities of water until it is thoroughly cooked in the form of a thick mush. It is then put into the remainder of the water or bouillon and subjected to streaming steam for two hours, after which, if the first heating was sufficient, it filters readily without the use of a hot-water filter, or the necessity of keeping it in the steamer during the filtering. The stirring rod must touch all parts of the bottom of the dish exposed to the flame, every few seconds during the preliminary heating, otherwise the agar will burn on and be spoiled. On some accounts it is best to begin operations with beakers rather than the enameled iron dishes. In this way all likelihood of using burned agar is avoided, since the moment the agar burns on the beaker cracks and the agar is spilled. For bacteriological use agar should be clear, not cloudy or filled with unremoved precipitates.

The writer now employs an autoclave and uses an agar flour procured from Lautenschläger or Merck (fig. 33). If one has an autoclave the preliminary heating of the agar in an open dish with a minimum quantity of water and all the subsequent stages may be dispensed with and the entire process carried on in the autoclave, unless it is known or suspected that media heated in the autoclave are less well adapted to the growth of particular organisms than those prepared at 100° C. The amount of agar added to the culture fluid is usually 1 per cent. On the making of nutrient agar consult "Formulæ," and the various standard text-books.

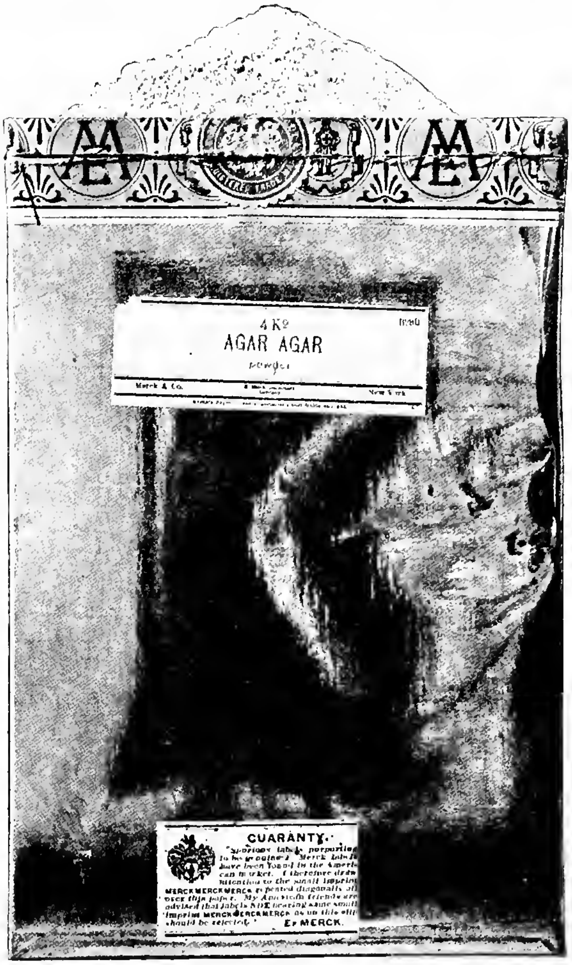

Fig. 33.*

Is there any difference in the appearance of colonies when grown at 5° to 10°, 15° to 20°, and 30° to 37° C.? Observe the amount of precipitate that collects in the fluid in the **V**. For other observations as to growth on this substratum see "Gelatin." Every organism should be studied in numerous Petri-dish poured-plate cultures. Too many plate cultures can scarcely be made. Dishes with flat and very thin bottoms (0.3 mm.) are desirable for some purposes, but are difficult to procure. For quantitative work, plates with flat bottoms are necessary, and when photographs are likely to be wanted plates must be selected which do not have rings, wavy places or other flaws in the glass on the bottom. There is room for much improvement in the quality of the Petri dishes now on the market.

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*Fig. 33.—Agar-agar flour as received from European manufacturers. Package of Merck's agar flour.

The student is advised to use agar media for all general laboratory work. When he has learned the behavior of an organism on nutrient agar, he may then try gelatin. Do any of the organisms under observation soften or liquefy the medium?

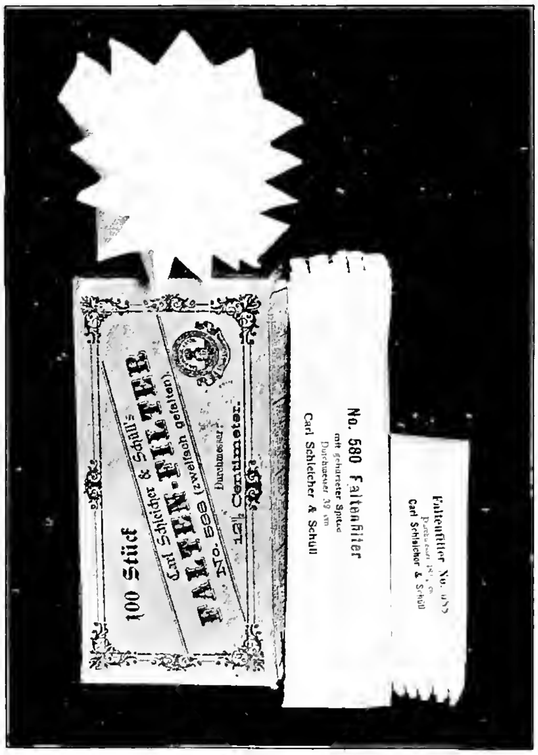

Fig. 34.*

Agar roll cultures may be made in test tubes readily if the amount of fluid agar is reduced to one-half cubic centimeter.

When colonies are to be counted, special pains must be taken to distribute the gelatin or agar uniformly over the bottom of the dish.

Various persons — Pake, Jeffer, Weiss, Macé, et al.—have devised ruled plates for counting the number of colonies of bacteria in Petri-dish poured plates. The writer prefers to count by square centimeters or fractions thereof. When the plate is sown thin enough, the entire number of colonies should be counted. When it is very dense, the average may be taken of ten square centimeters selected with care, provided the bottom is flat, otherwise the whole plate must be counted. If the counting plate is to be placed under the dish, it may be opaque, *i. e.*, a black surface with white lines, not the reverse. If it is to be placed on top of the dish, the latter preferably bottom up, then it should be of glass or some other transparent substance. The spaces may then be ruled on with a diamond, or drawn on in very fine black lines with India ink. The gelatin film of an unexposed, fixed photographic dry-plate is a very good surface for holding the ink. For counting colonies on very densely sown plates, the writer has found convenient a rectangle 20 mm. by 5 mm. divided into tenths.

SILICATE JELLY.

In recent years, in the hands of Winogradsky and his students, silicate jelly has played an important part in the isolation of various organisms, which do not take kindly to culture media containing animal and vegetable products. It is desirable also for exact experiment with other organisms. It may be used in Petri dishes or flasks, or slanted in test tubes. Along with some disadvantages, *e. g.*, tendency to split, it has a number of valuable characteristics, not least among which is the fact that it enables one to offer the organism a solid substratum which is at the same time purely synthetic. It is generally considered to be very difficult to make, but by following the most recent directions of Omélianski ('99, Bibliog., XXV), and especially certain slight modifications introduced by Moore & Kellerman and by the writer and his assistants, it can be prepared without difficulty, and to it may be added any mineral nutrient substances desired. The writer makes it in the following way:

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*FIG. 34.—Folded filter papers made by Schleicher & Schüll.

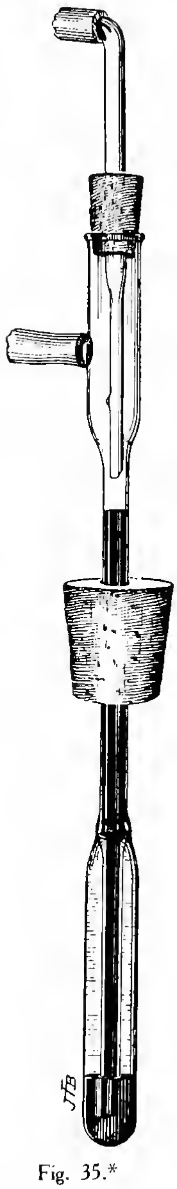

Fig. 35.*

To each 100 cc. HCl (sp. gr. 1.10° Beaumé) is added drop by drop 100 cc. sodium silicate (sp. gr. 1.09), the mixture being stirred continually with a glass rod. This is now placed in a collodion sack and dialyzed for some hours in running water. To this is then added in concentrated sterile form whatever synthetic culture medium is desired, after which the jelly is put into Petri dishes or test tubes and sterilized by heating for three hours in the blood-serum oven (fig. 45) on five consecutive days at 90° C., or by one steaming in the autoclave for 15 minutes at 110° C. The thermo-regulator shown in fig. 35 is useful for maintaining a constant high temperature in the oven. The oven must also contain some water in a capsule or beaker.

It is believed that a more detailed account of the manipulations connected with the preparation of silicate jelly will be welcome to many. First of all, one must have dialyzing sacks. Collodion sacks are much more convenient than parchment sacks, since they can be prepared at any time, and dialysis takes place through them with great rapidity. They are useful for so many purposes that material for making them should be on hand in every laboratory.

The writer follows Kellerman in making his sacks *inside* of test tubes. These may be large or small according to what the sacks are to be used for. If for dialyzing silicate jelly in some quantity, it is very convenient to make the sacks inside of test tubes 7 inches long and having an internal diameter of 1 inch. The first thing is to prepare the collodion mixture. This is made by dissolving soluble guncotton, such as is used by photographers, in a mixture of absolute alcohol and sulphuric ether. The writer uses equal parts of these two fluids. If too much alcohol is used, the sacks dry slowly, and if too much ether they are said to become brittle. After some experimenting it was found that 5 grams of the clean, white guncotton per 100 cc. of the fluid gave a solution very satisfactory to work with. About 24 hours is required to dissolve the guncotton into a homogeneous mixture, of which there should be at least 800 cc. This should be stored in a cork-stoppered bottle of shape convenient to hold in one hand. It is then ready for use. The clean test tube, thoroughly dry on the inside, is now held in one hand in a slanting position, mouth up, while with the other the collodion is poured slowly and steadily into the tube, while the latter is slowly rotated. In this way air-bubbles are avoided and the entire interior of the tube is moistened. When this has taken place and about an inch of fluid has accumulated in the bottom of the tube, the excess is poured back into the bottle, slowly rotating the slanted tube, as before, so as to cover again the entire interior with as uniform a layer as possible. When the bulk has been poured back, the tube is stood upright, mouth down, to drain on a sheet of clean paper. In two or three minutes it will have drained sufficiently, the excess of accumulations about the mouth being wiped off on the paper now and then. The tube is then seized and rotated in a horizontal position for four or five minutes with the mouth in the draft of an electric fan, or the rotation may be somewhat longer if no air-current is available. A little experience will tell when the sack is dry enough to remove from the tube. The strong smell of ether must have somewhat subsided and the collodion must not feel wet around the mouth of the tube, as will be the case if the layer of collodion is too thick in places. If it is taken out in this condition, the thick, wet places will become clouded. The collodion is now cut free at the lips of the test-tube by means of a pin-point or other sharp instrument and the tube is filled with cool water, taking care to let it also flow between sack and wall of tube if there is any shrinkage. In a minute or two, if the work has been well done, the sack, free from air-bubbles and filled with water, may be readily lifted out of the tube. It is then placed in a jar of water, where it remains until it is ready to receive the substance to be dialyzed. These sacks are quite tough, and there is little danger of tearing them during filling and tying.

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*Fig. 35.—Tollen's thermo-regulator for maintaining blood-serum oven at 80° to 90° C. The stem and bottom of the bulb contain mercury. The remainder of the bulb is filled with glycerin. In the similar thermo-regulator used for the paraffin-bath chloroform replaces the glycerin. Actual height, 12 inches. Chloroform and glycerin are very useful in such thermo-regulators because their coefficient of expansion is much greater than that of mercury. Toluene may also be used with mercury.

When the silicate jelly or other substance has been placed in them, the mouth is brought together and tied by means of a small rubber band, the elasticity of which keeps the sacks perfectly tight. Silicate jelly should be dialyzed for at least 12 hours, and sometimes for 24 hours, if every trace of salt must be removed. The writer fills the sacks with the silicate jelly in the afternoon and leaves them in running tap water over night. The next morning they are taken out, their contents emptied into a clean beaker, the nutrient salts added, and the fluid immediately pipetted into tubes, flasks, etc., and sterilized by heat. The nutrient substances should be dissolved in advance, so as not to delay the preparation of the medium. They should be added for this purpose to a minimum quantity of water. Some dissolve slowly, and there is a preferable order of solution, the glycerin being added last in case of Fermi's solution.

For the preparation of silicate jelly a Beaumé hydrometer for liquids heavier than water is used. C.P. hydrochloric acid of any specific gravity is diluted with distilled water until it tests 1.10° on the scale of the hydrometer when cooled to 60° F. Clear homogeneous sodium silicate of any specific gravity is then mixed with distilled water until it is of sp. gr. 1.09° Beaumé at 60° F. A great deal of water must usually be added to the sodium silicate, and the first dilution is tedious. For example, 100 cc. of a sodium silicate of sp. gr. 1.42° required the addition of 750 cc. of distilled water to give a fluid registering 1.07 Beaumé. On adding the fluid containing the nutrient salts, and hardening, sodium silicate of sp. gr. 1.07° Beaumé gave a rather too fluid medium, and sodium silicate of much higher sp. gr. than 1.09° Beaumé is apt to set before it has properly dialyzed, or after adding the nutrient salts and before it can be tubed and slanted. Several liters of the diluted acid and sodium silicate may be conveniently made up at one time. When these are ready, equal volumes of the two are mixed. This is done by adding the sodium silicate drop by drop to the acid, rather rapidly, stirring meanwhile with a glass rod. The top part of the apparatus shown in fig. 146 may be used for this purpose. The salty, acid fluid is now ready to be placed in the collodion sacks for dialyzing in running water. It is ready for removal from the water when it is no longer acid to litmus and shows only traces of sodium chloride remaining. An exposure to the running water for 6 hours is scarcely sufficient, unless the sacks are small.

For many purposes Fermi's solution is a good one to add to the dialyzed jelly. This is made as follows, for this purpose: Freshly-boiled distilled water, 100; magnesium sulphate, 0.2; monopotassium phosphate, 1.0; ammonium phosphate, 10.0. Dissolve. Then add glycerin, 45.0.

The dialyzed silicate jelly is now poured out of the collodion sacks into a clean beaker and brought to a boil for a minute or two over an open flame (to drive off the absorbed air). It is now cooled down to 50° C. and the Fermi added. If this has been dissolved over night it must also be brought to a boil and cooled, or have the air removed under an air-pump before adding it to the silicate jelly. To 500 cc. of the dialyzed fluid, 90 cc. of the Fermi may be added. This is stirred with a clean glass rod and then quickly pipetted into test tubes.

It is now placed in the autoclave without delay in the position desired and heated for 15 minutes at 110° C. To avoid tearing the surface of the jelly by steam, the autoclave must be carefully shut steam-tight as soon as the air is driven out, and it must not be opened until the temperature has again fallen to 100° C. It is also necessary to keep the autoclave closed on account of loss of ammonia from the ammonium salt. For this reason it is desirable to dissolve the Fermi in freshly-boiled water and to pump out any absorbed air rather than to boil it out.

Other nutrient salts may be added—Uschinsky's solution, etc. The writer has had very good success with Fermi for differential purposes. Many organisms grow remarkably well on this substratum, while others do not vegetate, or make only a scanty growth.

The observations on this medium are the same as for gelatin or agar. Observe character of growth, staining of substratum (green, pink), etc.

Solid Vegetable Substances.

These should consist of slant cylinders in cotton-plugged test tubes half covered with distilled water and steamed 20 minutes at 100° C. on each of three consecutive days. The addition of considerable water enables one to keep the culture under observation for several months without danger from drying out if the cotton plugs are properly made. Drier culture media may also be used. If one wishes to do so, the potato or other substance may be lifted entirely out of the water by making a constriction in the lower part of the test tube, à la Roux, or by thrusting a wad of absorbent cotton into the bottom of the test tube before the potato is introduced. The writer has not found these methods necessary. In general, I prefer vegetable media which have been sterilized in the steamer rather than in the autoclave.

The following are some of the vegetable substances recommended:

(1) Potato.
(2) Sweet potato.
(3) Carrot.
(4) Sugar-beet.
(5) Turnip.
(6) Radish.
(7) Salsify.
(8) Parsnip.
(9) Onion.
(10) Banana.
(11) Coconut.
(12) Peanuts.
(13) Brazilnuts.
(14) Apple.
(15) Pear or quince.
(16) Pineapple.

These substances may be extended almost indefinitely and are very useful for making preliminary studies, inasmuch as they include many different kinds of chemical substances. The writer has used them for many years. They should be prepared with great cleanliness, especially the roots, so as to avoid including resistant spores. Sterilization is an easy and simple process if these substrata are free from spores when the steaming begins. Roots and tubers should be selected with great care, only those being taken which are sound and free from blemishes. They are now to be washed thoroughly in tap water with scrubbing and then rinsed in distilled water. With clean hands and a clean knife they are then pared, with care to remove all black specks, and thrown into a beaker of distilled, filtered or boiled water. Cylinders of the proper size may now be punched with a clean cork-borer or cut with a clean sharp knife and, after the upper part has been slanted, are thrown into another beaker of distilled water, from which they are transferred to two others before they are finally put into the tubes. It is not necessary to soak them in water over night or in antiseptic solutions. They will not brown by oxidization if they are kept under water during the early stages of preparation and are steamed as soon as they are placed in the tubes, *i. e.*, exposed to the air. They may be put into the tubes with clean fingers or by means of a pair of clean forceps.

On these different media observe the nature, amount, and rapidity of growth (always with due regard to the air-temperature, which should be recorded). Carefully determine whether there is any retardation of growth at first and, if so, to what it is due, so that more exact studies may be made subsequently in other media. Look for gas-bubbles, formation of acids and alkalies, formation of hydrogen sulphide, of crystals, of stains, of odors, destruction of starch, disappearance of the middle lamella, softening of cellulose, etc. For the first few days all cultures should be examined at least as often as once in 24 hours and, generally speaking, cultures should not be discarded until after the sixth or eighth week. These experiments should be repeated a number of times and the student should avoid drawing a hasty conclusion, since different samples of potatoes, carrots, etc., vary somewhat in composition and will at times give slightly varying results or even results which seem to be contradictory, *e. g.*, a brown pigment in some instances and not in others.

The same media, and as many other sorts as are available, should be tested raw in sterile, dry, Petri dishes 10 cm. broad and 2 to 3 cm. deep. For this purpose the vegetables are prepared as follows: First, select sound, clean specimens, especially avoiding those which are cracked open; next, scrub their surface thoroughly under the tap, and rinse them in distilled water. They are now soaked 5 or 10 minutes, or even 20 minutes, in 1:1000 water solution of mercuric chloride. They are then removed and dried with or without a preliminary rinsing in sterile water. When dry they are put on a sterile paper or plate, are cut into slices about 1.5 to 2 cm. thick with a cold sterile knife, are picked up with sterile forceps, and are put into the Petri dishes in pairs or fours, the cover being immediately replaced. Enough of the mercuric chloride remains on the surface to inhibit the growth of any surface organisms which have not been killed outright, and experience shows that intruders are rarely dragged over the cut surface. The slices may be inoculated at once or after 36 hours incubation in a moist chamber at 30° C., or 48 hours at 25° C. The latter course is preferable. In either case, half of the slices in each dish must be kept uninoculated for comparison (fig. 36). This method is well adapted to the study of various soft-rot organisms such as *Bacillus carotovorus*, *B. aroideæ*, *B. oleraceæ*, etc.

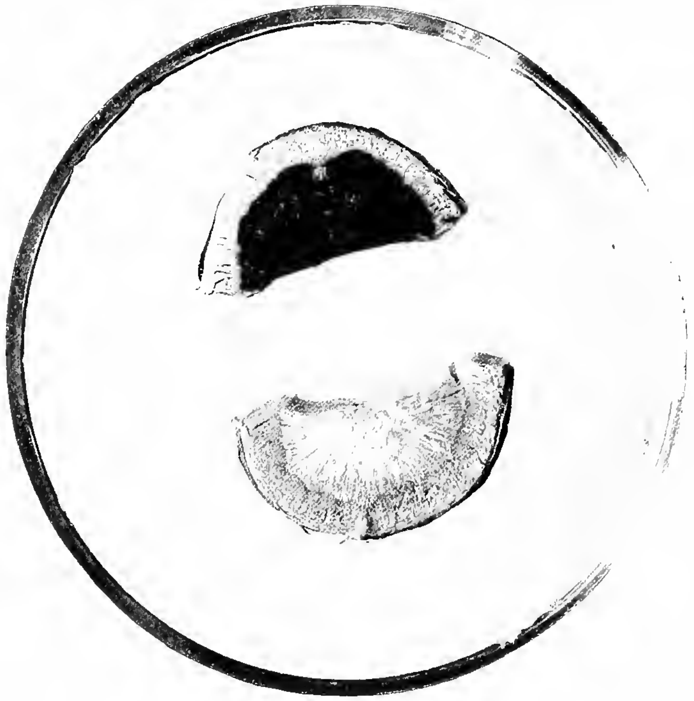

Fig. 36.*

PLANT JUICES (WITH AND WITHOUT THE ADDITION OF WATER).

(1) Juice of the host-plant.
(2) Potato-broth.
    (*a*) With sodium hydrate.
    (*b*) Without alkali.
(3) Cabbage or cauliflower broth.
(4) Prune-juice.
(5) Orange-juice.
(6) Coconut-water (unsteamed).†
(7) Yellow corn-meal broth.
(8) Timothy-hay infusion.

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*Fig. 36.—Iris-rhizome-rot organism grown on raw carrot. The check piece is unchanged, the inoculated piece has browned and softened. Incubated 4 days at about 23° C.

†This is removed directly from the nut to sterile test-tubes by means of sterile pipettes, which are useful in many ways. The pipettes should be dry-heated and kept from contamination in long, narrow, covered tin boxes. These boxes may be cylindrical or rectangular, with an end cover. The upper end of the pipette should be plugged firmly with cotton before sterilization, and this should be pushed in a short distance beyond the end, so that when the finger is placed on the end there will be an air-tight union. Scalpels, etc., should be sterilized in shorter boxes of the same kind (fig. 37).

These fluids are only a few of many that may be used. Some of them, *e.g.*, potato-broth, require special care in preparation. My own method of making potato-broth is to pass the clean pared potatoes rapidly through a grating machine and immediately throw the pulp into the required quantity of distilled water (which should be twice the weight of the potato). The beaker is now put into a water-bath and the temperature rapidly raised to 55° C. and kept there with frequent stirring for an hour. The pulp is now filtered from the fluid and the latter is immediately put into the steamer. If the steaming is long delayed the broth will be dark brown (oxidizing action of the potato-enzyme on tannins in the presence of air), and if the temperature rises much above 60° C., before the pulp is removed, some of the starch becomes gelatinous and the fluid will not filter.

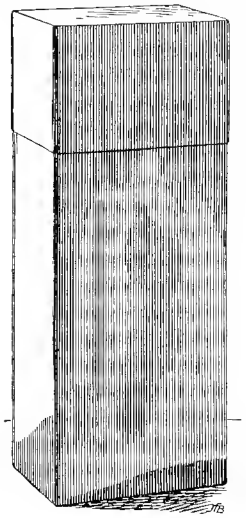

Fig. 37.[*]

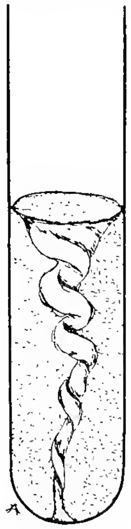

Fig. 38.[†]

All media which have boiled away to any considerable extent must, of course, be made up to the original volume or weight just prior to final sterilization.

In these culture-fluids observe the rapidity, density, and *persistency* of the clouding; whether the clouding is simple or turbid from the presence of zoogloeæ; and finally, whether it is uniform in all parts of the tube. Note the character of the rim and pellicle, if any are formed, and how soon they appear; also the amount, color, and general appearance of the precipitate. The amount of the precipitate varies greatly with different media. Its quality also varies. Sometimes it consists of loose, easily separable particles; in other cases it is a viscid mass which rises as a rope-like unit when the tube is twirled (fig. 38).

Record the formation of acids, alkalies,[‡] odors, gas-bubbles, stains, crystals. Does the fluid become viscid or ropy? Some organisms bring about this condition quickly in a variety of media, *e. g.*, *Bacterium pericarditidis* (*Bacillus pyocyaneus pericarditidis*), others rarely or never. Precipitates in test-tube cultures vary all the way from a scarcely perceptible trace to masses a centimeter or more in depth. Do not confound chemical precipitates with bacterial growth. Before inoculation always examine media in test-tubes for presence of slight precipitates and for contaminating organisms. In cultures of rapidly growing species, at optimum temperatures, clouding may occur in less than twenty-four hours; with slow-growing species, and

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[*] Fig. 37.—Tin box for holding scalpels, forceps, etc., to be sterilized by dry heat. About one-fourth actual size. A similar tin box which is very convenient for holding sterile pipettes measures 2 by 3 by 15 inches.

[†] Fig. 38.—Twirled culture of the olive-tubercle organism in Uschinsky's solution, showing viscidity of the precipitate in old cultures.

[‡] Bacterial ash is alkaline, and this ash must be carefully washed from the platinum loop in distilled water each time before it is used to transfer drops of the culture-fluid to litmus paper. The wire must, of course, be re-flamed after washing.

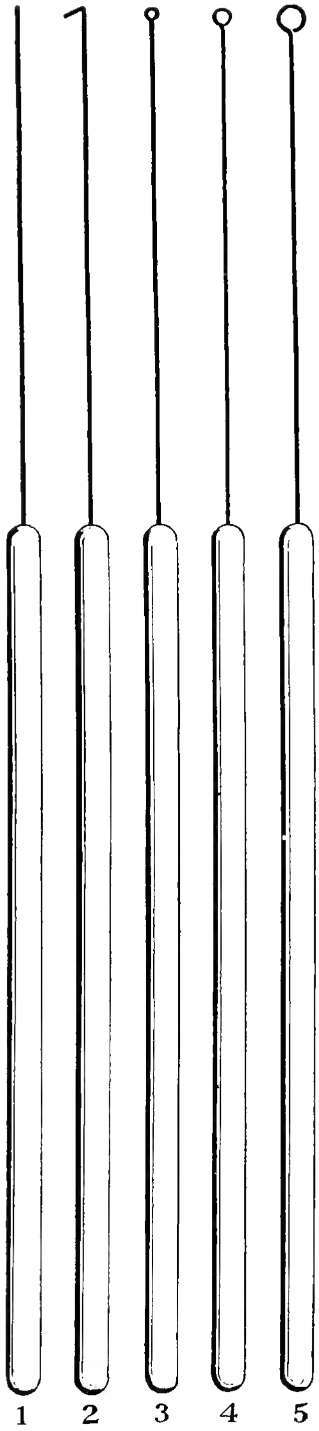

Fig. 39.†

when the medium has a retarding action, it may not occur until after two or three weeks. Of course, the rapidity of the clouding depends to a considerable extent on the size of the loop and on whether the inoculation was from a young or old, a fluid or a solid culture.

Among other tools, the student should be provided with five platinum-iridium wires set into glass handles, three of which are bent at the free end into loops of a definite size, *i. e.*, with an inside diameter of 1, 2, and 3 mm. These are made by bending around wires of the given size, and will enable one to measure out approximately uniform quantities of fluids and solids. Smaller quantities may be transferred on the extreme tip of a straight platinum needle. It is also convenient to have a platinum* needle bent at the end into a short hook (see fig. 39). In comparing rates of growth in fluid cultures it is best to inoculate them from other fluid cultures of a given age and not from solids.

If there is any reason to think that boiling changes the nature of any of these fluids, they should be sterilized cold by forcing them through a Chamberland or Berkefeld filter. The Chamberland has the finer pores, the Berkefeld filters quicker. The simplest way of using such a filter is that first described by Dr. Theobald Smith, viz, to put the fluid inside and force it out by means of clean compressed air. For this purpose select a flat-bottomed cylindrical glass vessel (a round-bottomed one is less convenient, but may be set into a hole bored in a block of wood) of a larger diameter and 5 or 10 centimeters longer than the bougie, which should be clean (previously unused), but washed by having had some liters of distilled or filtered water forced through it. Wrap the nipple-end of the filtering cylinder firmly with clean cotton for a distance of 5 or 10 cm. down. Thrust the wrapped bougie into the glass vessel securely, so that only the nipple and the cap or shoulder projects. The top of the bougie should also be wired so that it can not possibly slip down during the filtering. This apparatus should now be sterilized by putting it into the dry oven for two hours at 145° C. Wrap in clean Manila paper and heat at the same time a large cotton plug, *i. e.*, one which has been made to fit the mouth of the cylindrical glass vessel. When sterilized and ready for use, select a piece of rubber cloth 10 or 15 cm. in diameter, cut a small slit in its center and draw it over the nipple of the bougie to protect the cotton from accidental wetting and the filtered fluid from consequent possible contamination. Now pour the fluid into the bougie (if one with a large neck has been selected this will not be difficult, especially if a small funnel is used and this is kept from close contact on one side by means of a small wire, sliver, or bit of paper), and connect the nipple with the outflow-tube of the compressed-air pipe by means of an *extra-thick* rubber tube, which should be securely wired at each end, and turn on the compressed air cautiously. Fluids which are not colloidal usually filter very readily with a pressure of 15 or 20 pounds per square inch.

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*Platinum-iridium is preferred to pure platinum because it bends less easily. The wire used by the writer has a diameter of 0.48 mm. The alloy as usually found on the market is said to contain about 10 per cent of iridium, sometimes less, but never more. The wire shown in fig. 39 was made to order and contains 20 per cent iridium.

†Fig. 39.—Platinum-iridium wires set into glass rods, for bacteriological work. 1, needle; 2, hook; 3, one-millimeter loop; 4, two-millimeter loop; 5, three-millimeter loop. The size of this wire is about one fifty-fifth inch.

The filtering should always be done slowly with a minimum pressure in order to avoid the possibility of forcing small organisms through the walls of the filter. With heavy pressure this sometimes occurs when no cracks are detectable in the bougie. When the desired quantity of fluid has been filtered (fig. 40) cut off the air-blast, disconnect the tube, tilt the cylinder as much as possible, remove the bougie, and substitute the sterile cotton plug. The fluid should now be transferred *immediately*, in 5 or 10 cc. portions, to sterile cotton-plugged test-tubes by means of sterile pipettes. The removal of the bougie and the transfer of the fluid should be done in clean still air, under a hood or in a special culture-room. The tubes should not be used for several days, *i. e.*, time should be given for contaminations to show themselves, but if proper care has been exercised there should be very few contaminations or none at all. A pressure much greater than 20 pounds per square inch may be obtained by means of steam-pumps or by use of cylinders of compressed air, oxygen, or carbon dioxide, and this is sometimes necessary for colloidal substances, but should be used cautiously. These cylinders may be had from the Eagle Oxygen Company, New York. One of the most convenient filters on the market is that shown in fig. 41. It was designed by Roux and is made by Maison Wiesnegg (P. Lequeux), Paris. It is well made, very durable, quickly sterilized, and easily operated if one can command an air-blast or other gas-pressure of 2 or 3 atmospheres.

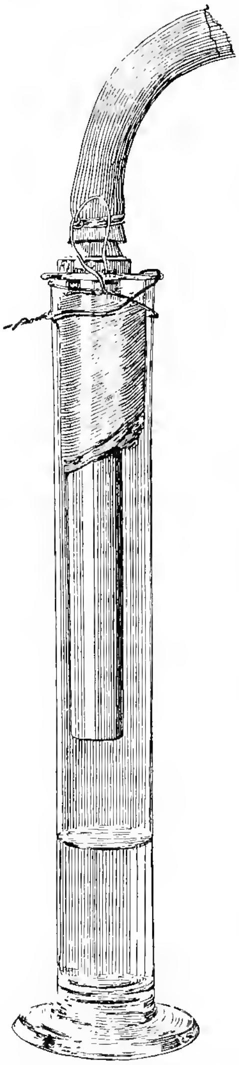

Fig. 40.*

Chamberland bougies ought not to be used continuously for more than three days. They should then be removed and baked for two hours at 145° C. (or at the temperature of an oven in which bread is baked). The reason for this lies in the fact that in three or four days time certain small organisms are able to grow through the walls of the filter and make their appearance in the filtered fluids on the other side. Persons who never bake their water-filters rest in unwarranted security. The bougies must also be handled with great care and inspected carefully after each baking for the appearance of minute cracks. To detect cracks, immerse the tube in water and blow into it. Clogged filters should be sent to the firers of china, where they may be purified by heating to dull redness.

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*Fig. 40.—Simple method of obtaining small quantities of sterile fluids by means of the Chamberland filter. The other end of the rubber tube is wired securely to the outflow pipe of the compressed-air system and the fluid is forced from the inside of the filter out. This method was first described and figured by Dr. Theobald Smith. About one-fourth actual size.

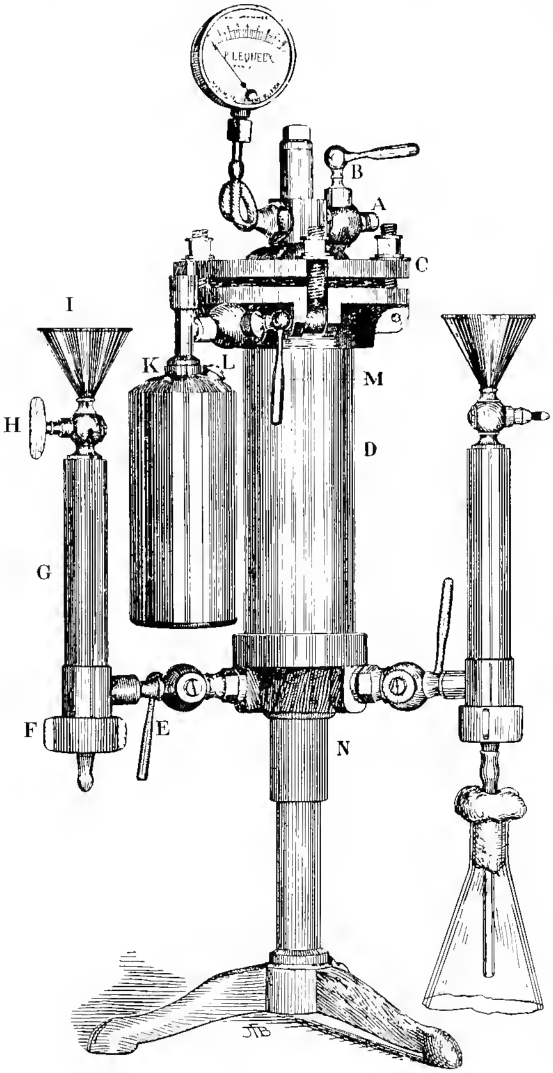

Fig. 41.*

Animal Fluids.

BEEF-BROTH.

(*a*) Acid, neutral, and alkaline.

(*b*) The same, with addition of 0.5 per cent c. p. sodium chloride and 1 per cent peptone (Witte's peptonum siccum, Merck's brown peptone, Savory & Moore's brown peptone, etc.). This is ordinary peptonized beef-broth.

Examine as in case of plant juices. The term peptone, as it occurs in bacteriological literature, usually means commercial peptone, which is a mixture of true peptone and various proteoses or albumoses. It is therefore generally best to specify just what peptone is used. The writer now generally uses Witte's dry white peptone. Savory & Moore's brown peptone from flesh is very good for some purposes.

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*Fig. 41.—Dr. Roux's pressure-filter, made by Maison Wiesnegg (P. Lequeux), Paris. The working capacity of this filter is about 1.3 liters. The principal parts are: A, tube for connection with compressed-air system; B, cut-off; C, cover held in place by strong bolts; D, central reservoir; E, cut-off; F, screw collar which holds the bougie in place; G, heavy metal cylinder surrounding the bougie; H, cut-off, which is closed of course when the apparatus is in use; I, funnel through which G and D are filled; K, device for sterilizing the interior of the apparatus by steam under light pressure (it consists of a copper chamber partly full of distilled water, to the bottom of which the Bunsen flame is applied; the chamber may be unscrewed and removed); L, button which is unscrewed to fill the chamber with water (in its center is a steam safety valve acting under feeble pressure); M, valve which cuts the steam-generator out of the general circulation when fluids are being filtered; N, tripod-top on which the apparatus turns freely. Height, 33 inches.

Milk.—Milk from a clean dairy and free or nearly free from cream should be selected for use. If some cream remains it may be filtered out or removed by the centrifuge (fig. 43). The milk should not be acid to the taste and should not contain formaldehyd or other antiseptic substances which milk-dealers sometimes add to dirty milk to improve its keeping qualities. It should be steamed in wire-crates 15 minutes at 100° C. on each of four consecutive days (10 cc. portions in test-tubes), and should not be used until at least a week after the last steaming. Such milk should titrate + 12 to + 17 or thereabouts with sodium hydrate and phenolphthalein. Milk-cultures should be kept under observation at least six or eight weeks.

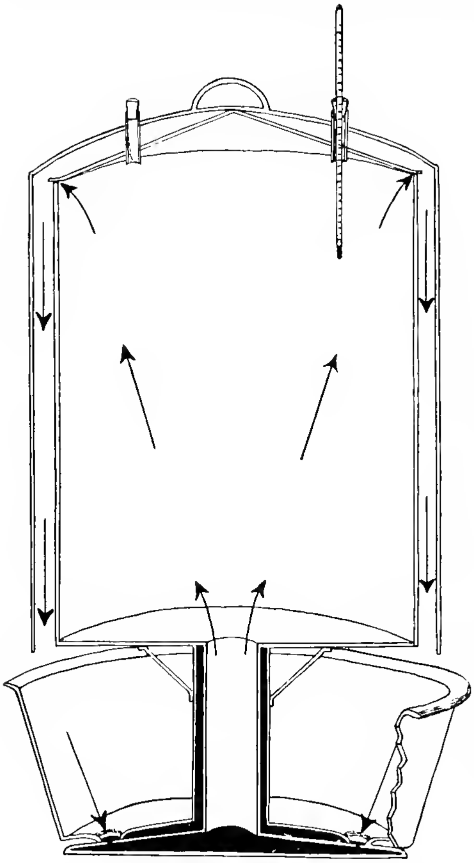

Fig. 42.*

Observe in particular:

(*a*) Separation of the casein without the development of any acid, indicating the presence of the lab, or rennet, ferment. The milk usually becomes more alkaline.

(*b*) Saponification of the fat. The fluid becomes transparent without any precipitation of casein; but the caseinogen may be thrown down subsequently by acidifying the clear liquid.

(*c*) Ropiness. The fluid becomes viscid, and strings when touched. This viscidity is sometimes so great that an entire pail of milk may be inverted without immediate loss of its contents. See striking figures in Ward's papers ('99 and '01, Bibliog., XLVII).

(*d*) Formation of acids. This occurs with or without evolution of gas, and usually with the final separation of the whey from the casein at room temperatures or on boiling. Boil if necessary.

(*e*) Re-solution of precipitated casein (trypsin ferment); formation of crystals (tyrosin, leucin, etc.).

(*f*) Gelatinization of old cultures. Milk alkaline.

(*g*) Changes in smell, color, and taste.

In using milk it should not be forgotten that anaerobes are sometimes present (Theobald Smith) and also organisms of the dunghill which will grow only at temperatures above 40° C. Very resistant spores of aerobic species, growing at temperatures below 40° C., are present also sometimes, especially in dirty milk, and the milk is then difficult to sterilize.

Several experiments made by the writer with milk from Washington dairies have shown that Franz Lafar's statement in Technische Mykologie, Bd. I, p. 189, while probably true for the milks which he tested, is not true when stated as a general proposition. In brief, this statement is that nine out of ten milks are not sterilized by steaming twenty to thirty minutes on three consecutive days, but will develop bacterial growths when put into the thermostat. If such were really the case, milk would be one of the worst of culture-media instead of one of the best. The general experience of bacteriologists is not in accord with this statement. Occasionally, in my own experience, a single steaming of five or ten minutes has sufficed to sterilize milk completely, at least so far as relates to organisms which grow aerobically and at temperatures under 40°C. Such milks have remained unchanged for two or three months at room temperatures (20° to 25° C.), and also in the thermostat at blood heat. For anaerobes, or organisms which will grow only at temperatures above 40° C., I have not tested.

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*Fig. 42.—Section of Arnold steam sterilizer. Water enters the double bottom through a few small openings indicated by two arrows in the water-pan. The other arrows show movement of the steam. In this form the outer jacket (of copper) is lifted off to put in or remove media.

One possible source of error in the use of steam for sterilization is ignorance of the exact temperature of the steam-chamber. Every steam-sterilizer should have a hole punched through the top, into which is fitted a cork through which a thermometer projects into the chamber. In this way may be determined beyond doubt for just how many minutes the media has been exposed to steam at 100° C. The Arnold steam-sterilizer, which is one of the best,† is greatly improved by this simple device (fig. 42 and pl. 6). In this sterilizer there is a double bottom under the water-pan. The lower bottom is in contact with the Bunsen flame. Through small holes in the upper

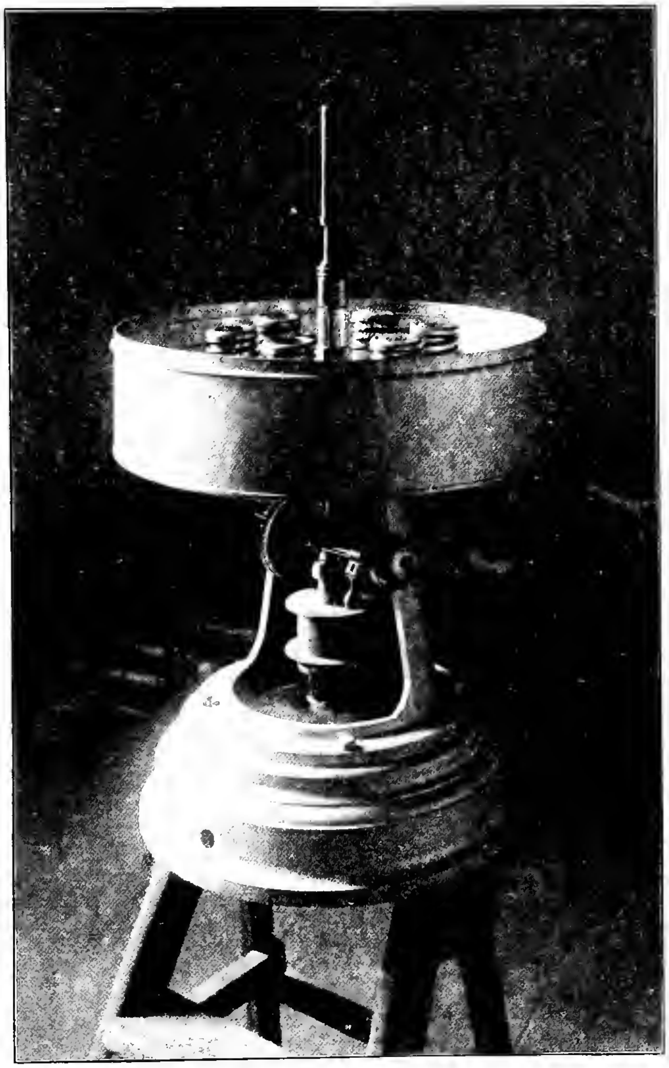

Fig 43.*

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*Fig. 43.—Improved Lautenschläger centrifuge. Capacity, 540 cc. Revolutions per minute, 3,000 to 4,000. It requires about 3 horsepower to run the apparatus at this high speed. About one-eleventh natural size.

†This remark does not apply to the Arnold combination steamer and dry oven, which can not be recommended.

bottom the water drips to the lower bottom and is quickly converted into steam which streams through a central chimney into the bottom of the sterilizing chamber. The latter has two walls, with a considerable air-space between, open at the bottom. The streaming steam passes over the top of the inner wall downward into this air-space and escapes into the pan as condensation water. Theoretically this is a very perfect sterilizer, and it is so in practice when new, but not infrequently it leaks, and sometimes the openings in the upper bottom are too large or become clogged by mud. When in perfect working order it takes only a few minutes to get a temperature of 100° C.

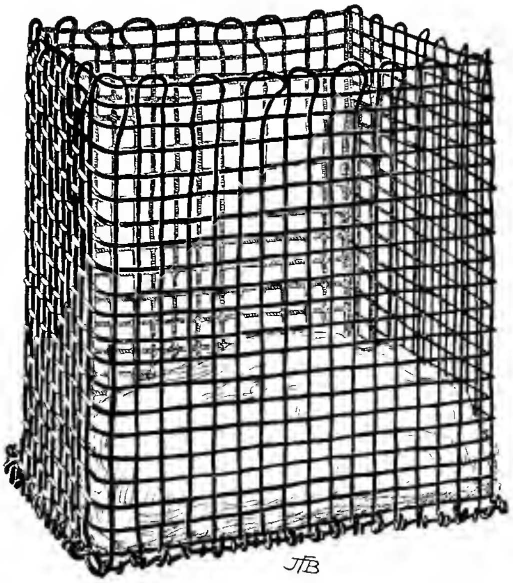

Fig. 44.*

Tubes should always be steamed in wire-crates (fig. 44) so that the streaming steam may have full access to all parts. Tubes of media steamed in cans or beakers often spoil. They seem to retain a cushion of air about them which interferes with the action of the steam.

Litmus milk.—Litmus milk of a good quality may be made by dissolving Merck's dry, lime-free c. p. blue litmus to saturation in distilled water (1:15) and then adding one part of this blue fluid to each fifty parts of milk. The milk should be a deep lavender color. Much inferior litmus is on the market. Large use should be made of this fluid. In addition to observations under "Milk," note how rapidly the litmus reddens, blues, or becomes reduced, and how soon the color returns. Will it return at once on steaming the culture?

Rice cooked in milk.—(One or two grams to 10 cc. in each test-tube). This is useful for study of some chromogens.

Loeffler's solidified blood-serum.—Observations under this and the following heads are the same as for gelatin slant cultures. The plant bacteriologist must in general obtain blood-serum from the animal bacteriologist. The solidified serum may also be used plain, *i. e.*, without the addition of grape-sugar.

Egg-albumen.—This is solidified and used in the same way as blood-serum. The end of the egg from which the albumen is poured must be thoroughly flamed before it is broken, and care must be used in the transfer to test-tubes so as to exclude air-borne germs as far as possible, otherwise the sterilization will be difficult. The albumen of eggs may be cut with sterile scissors.

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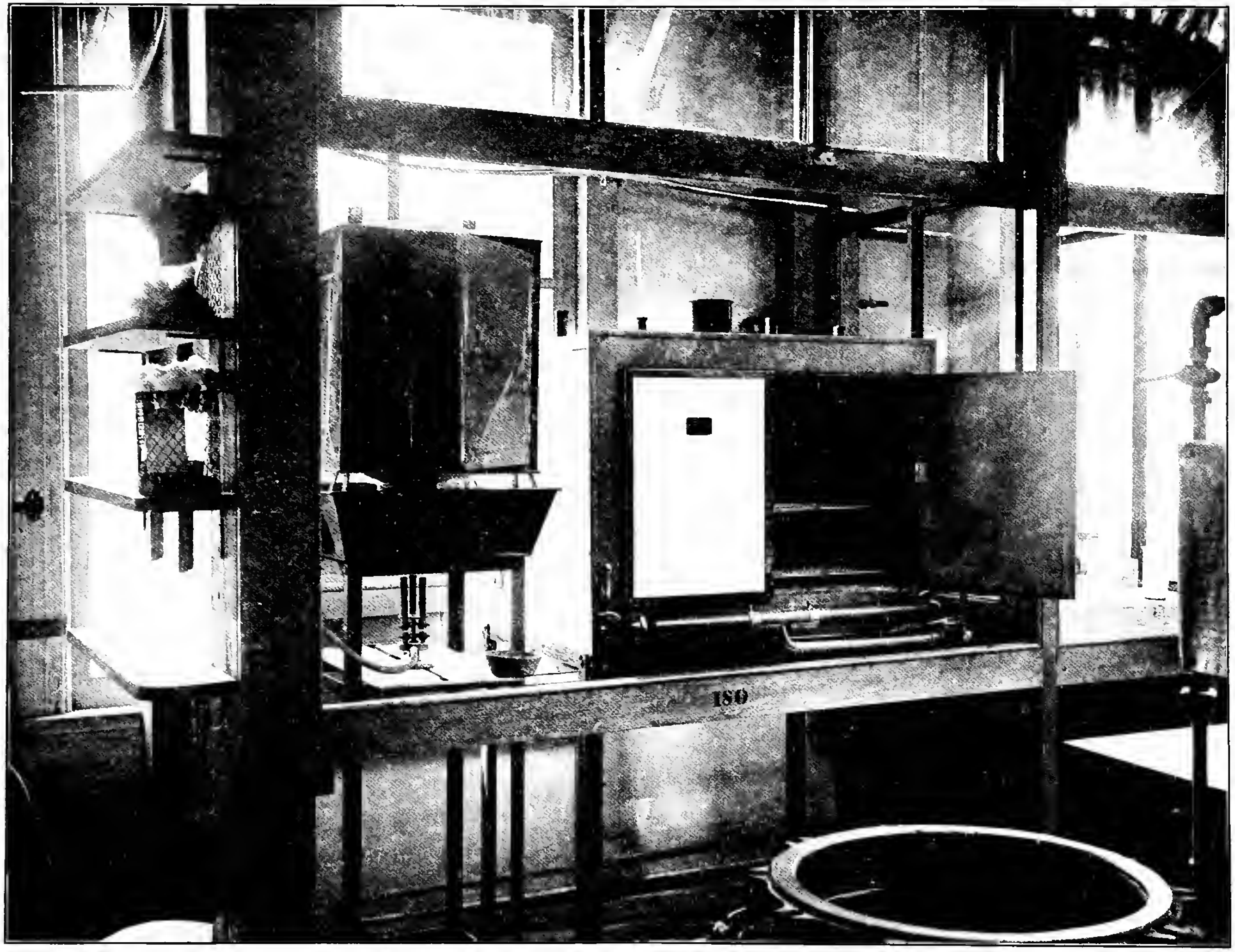
*Fig. 44.—Wire-crate for holding tubed culture-media which is to be steamed. About two-fifths actual size. A tuft of cotton on the bottom prevents the breaking of tubes.

PLATE 6.



Hood under which apparatus and culture-media are sterilized.

At the left is the Arnold steam sterilizer (Boston Board of Health pattern). At the right is the large size Lautenschläger dry hot-air sterilizer. In front, autoclave with cover removed.

Egg-yolk.—This is poured into test-tubes and solidified in a slanting position by heat (80° C.), or the egg may be boiled hard and the yolk cut with a sharp knife and transferred to sterile Petri dishes. If desired, the yolk and white may be mixed before solidifying, *i. e.*, by shaking the egg vigorously before breaking the shell.

SYNTHETIC MEDIA AND OTHER SPECIAL MEDIA.

The student should try the following media. He should also invent media to suit special cases. The kinds of media I have in mind are the opposite of universal, to-wit, such as will favor the growth of some organisms while preventing that of others. The acid phosphates and many other substances are useful for this purpose. The field is comparatively new, and much is to be learned by careful experimenting.

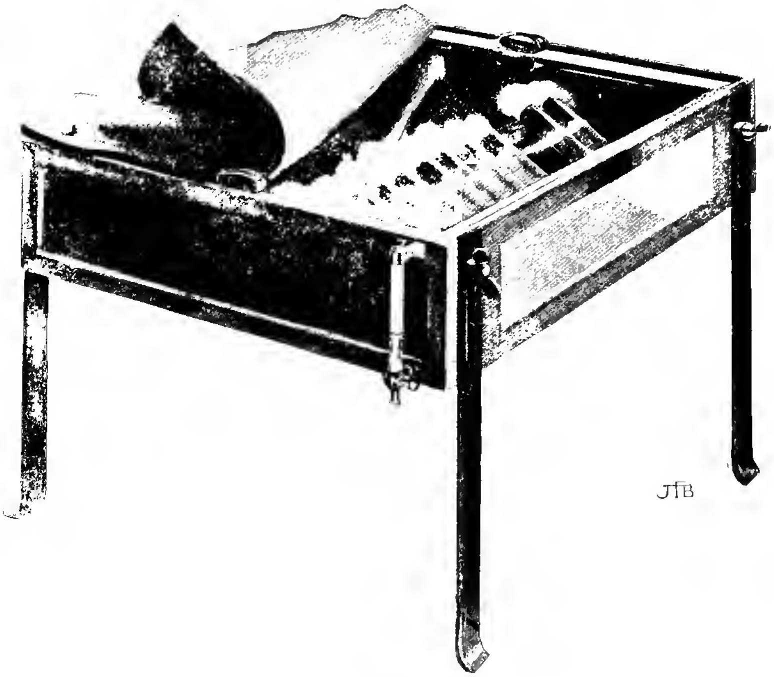


Fig. 45.*

1. Dunham's solution.
2. Peptone-water (1 or 2 per cent) with addition of various carbohydrates, acids, etc.
3. Sugar-free beef bouillon with Witte's peptonum siccum (for the indol test).
4. Cohn's solution.
5. Uschinsky's solution.

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*Fig. 45.—Oven for solidifying and sterilizing blood-serum, nutrient starch-jelly, silicate-jelly, etc., at temperatures below 100° C. When in use the temperature is controlled by means of a Tollen's thermo-regulator (see fig. 35).

6. Uschinsky's solution with various carbon compounds substituted for the glycerin (fermentation tubes).

7. Fraenkel and Voge's solution.

8. Raulin's solution.

9. Fermi's solution.

10. Water (distilled), 1,000,000 mg.; dipotassium phosphate, 2,000 mg.; ammonium phosphate, 100 mg.; magnesium sulphate, 100 mg.; sodium acetate, 5,000 mg.

11. Same, with the carbon compound changed, *e. g.*, with sodium formate substituted for sodium acetate. Sodium formate and phenolphthalein may be added also to bouillon or agar (2 per cent) for observations during the early stages of growth, some organisms reddening this medium promptly by decomposition of the sodium salt. (See a recent paper by Omélianski).

12. Nutrient starch-jelly for study of diastasic action. (See Proc. Am. Asso. Adv. Sci., 1898, p. 411, or Centralb. f. Bakt., 2te Abt., Bd. V., p. 102.)

One gram of starch is rubbed up with a sterile glass rod in 10 cc. of the sterile nutrient fluid (Uschinsky's solution, etc.), placed in a slanting position, in test-tubes, and solidified in a blood-serum oven (fig. 45) or in the top of a steamer with the vents left open. There should be several heatings of two hours each to insure sterilization. The temperature should not exceed 93° C. nor fall much below 85° C. Sterilization is rendered much easier if the starch is prepared in a cleanly way. The only difficulty the writer has experienced is in the formation of a thin film of semi-opaque solidified starch on the walls of the tubes above the slant. This often cracks off, however, during the heatings, and is largely obviated by placing the tubes in a slanting position before the starch is rubbed up in the fluid, taking care to soil the walls above the slant surface as little as possible during the operation. The potato-starch is prepared as follows:

One-half bushel of large smooth potatoes are scrubbed, and the black specks dug out; they are then soaked for 45 minutes in 1:1000 mercuric-chloride water. Meanwhile the hands are scrubbed clean and given a five minutes washing in the mercuric-chloride water. The tubers are now rinsed in sterile water, pared deeply, grated as for potato-broth, and thrown into beakers containing several liters of distilled water, where the pulp is worked over with the hands to liberate as much starch as possible. The starchy water is now removed from the pulp by passing it through several folds of surgeon's gauze, squeezing out of the pulp as much of the fluid as possible. When the starch has settled the brownish fluid and floating fragments are poured off or decanted, and fresh distilled water is added. The smaller fragments of cell-wall, etc., are then removed by forcing the starch (stirred up in water) through a moderately fine-meshed towel (not too fine) with gentle hand-rubbing, into another beaker. Most of the medium-sized and finer starch-grains pass through, leaving in the towel the coarser grains and those fragments of cell-wall which passed through the coarser meshes of the surgeon's gauze. The purified starch is now allowed to stand for about a week in the ice-box in distilled water (3 liters or more per beaker or jar). The water is siphoned off twice a day at first, and afterwards once a day, the starch being stirred up thoroughly every time fresh water is added. Finally the starch is drained very free from water, scooped out with sterile spoons or spatulas, placed in uncovered sterile Petri dishes, and dried in the blood-serum oven at 56° C., the cover being raised an inch (on corks) to let the moisture out. One-half bushel of sound potatoes should yield from 400 to 500 grams of air-dry aseptic starch.

Potato starch has been selected because it is easy to prepare, but other starches might yield interesting results. Bacteriologists now pay great attention to the fermentation of sugars, but thus far very little consideration has been given to the action of bacteria on starches and celluloses. Whatever starches are used, they should be prepared in the laboratory, under aseptic conditions, so as to exclude spore-bearing organisms.

13. Starch-jelly with addition of various sugars, gums, and alcohols (for study of organisms having little or no action on starch).

14. Tubes of slant nutrient agar (+ 15 of Fuller's scale) with varying amounts of c. p. glycerin, 2 to 10 per cent or more.

15. Tubes of 10 cc. slant agar with 10, 20, and 30 grams of grape-sugar.

16. The same, with the same amounts of cane-sugar.

17. Gelatin with cane-sugar, varying amounts.

18. Gelatin with malic acid. (17 and 18 may be combined.)

19. Gelatin plates with soluble starch and 1 per cent potassium iodide and with or without 1 per cent potassium nitrate. Try a mixture of the pear-blight organism and *B. coli*. Can the colonies be distinguished in this way using the nitrate?

20. Agar plates with various sugars and the addition of calcium carbonate, or zinc carbonate, for detection of acid-forming colonies. ('91, Beyerinck, Bibliog., XX.)

21. Silicate-jelly. See p. 36. Known also as silica-jelly.

22. Nitrate bouillon (+ 15 bouillon with 1 per cent potassium nitrate).

23. Triple-distilled water and nutrient mineral substances free from nitrogen. The same, with addition of potassium nitrate. The same, with other nitrogen foods, *e. g.*, sodium asparaginate.

24. Bouillon with lead acetate.

25. Bouillon with neutral red.

26. Salt bouillon, *i. e.*, + 15 bouillon with varying amounts of c. p. sodium chloride (1 to 5 per cent).

27. Standard peptonized bouillon with varying amounts of sodium hydrate (from + 40 to — 40) for determining the optimum reaction and the tolerated range of acidity and alkalinity.

Synthetic media may be varied indefinitely to fit special cases and are often extremely useful as differential tests. They have frequently been condemned because some particular organism has not grown *well* in them. The very fact of feeble growth or of no growth is, however, a matter of interest, and not infrequently a means of distinguishing organisms which resemble each other in many particulars. The value of such media becomes apparent at once when a number of organisms are compared. Synthetic media afford more exact methods of research than do the common media, and their value must increase rather than diminish as time goes on. (Consult Grimbert in Archives de Parasitologie, T. I, pp. 191-216.) It does not follow, however, that the common media should be at once abandoned. *Festina lente* is a good rule. The formulæ for some synthetic media are given under "Formulæ." For others see various text-books and the papers cited in the Bibliography under XVI, XVII, XVIII, XXV, etc.

RELATION TO FREE OXYGEN.

(1) Surface and deep growths.—Note the behavior of deep stabs in tubes of recently steamed gelatin and agar, or of the colonies in shake-cultures of gelatin and agar which are protected from the free action of air by pouring into the tubes as soon as solidified another tube of gelatin or agar in the surface layers of which, as an additional precaution, some active aerobe may be grown, *e. g.*, *Bacillus subtilis*. Observe also the relative rate of growth of buried and surface colonies in plate cultures, growth under sterile mica plates, etc. Of course, whether an organism will or will not grow under the conditions mentioned depends often to a large extent on the composition of the culture medium. It might be able to respire in the presence of grape-sugar or cane-sugar, but not when milk-sugar or glycerole is substi-

tuted. It will not do to conclude that an organism is a strict aerobe until it has been tested anaerobically in the presence of a variety of carbon foods with uniformly negative results. One who has had some experience may often give a shrewd guess as to behavior in fermentation-tubes by carefully noting the growth of buried and surface colonies in ordinary media.

(2) *Fermentation-tubes.*—The fluids may be Uschinsky's solution (without the glycerin unless this is the carbon compound to be tested); peptone water (2 per cent Witte's peptone with 0.5 per cent sodium chloride); and filtered tap water, or sugar-free beef bouillon with addition of 1 per cent Witte's peptone (preferably for most purposes this latter fluid). The substances to be tested (which should be chemically pure or as nearly so as possible) are grape-sugar, fruit-sugar, cane-sugar, milk-sugar, galactose, maltose, dextrin,* mannit, dulcit, raffinose, glycerin, ethyl alcohol,† methyl alcohol, acetone, ammonium lactate, ammonium tartrate, asparagin, sodium asparaginate, urea, etc. One to 5 per cent of the various sugars, etc., may be used; 2 per cent is a good quantity.

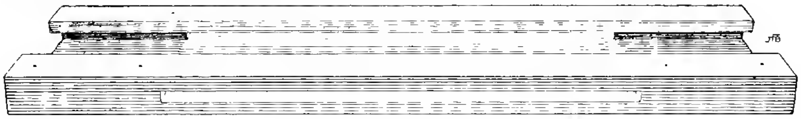

Fig. 46.‡

Observe carefully what substances induce clouding in the closed end and whether any gas is produced. Test from time to time for acids. The relative vigor of growth in the open end should also be noted. Does growth stop in the **U** with a sharp line of demarcation? Does the addition of calcium carbonate reduce or prevent the formation of gas or favor growth in any way? Is the reaction in the closed end, as the result of growth, different from that in the open end? Pipette out all the fluid from the open end, determine its reaction to litmus, and then test the reaction of that which remains. How is the difference, if any, accounted for? If growth finally ensues in the closed end, is there any reason for thinking it due to absorbed air? How can this be determined?

It should be remembered that often, after a time, air is absorbed into the closed end of fermentation-tubes and may lead to confusing results. For this reason, if they have stood on the shelf any length of time after sterilization, they should be re-steamed and the bubble of air tilted out before they are inoculated. They

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*The dextrin should be freely soluble in cold water and should not give any red reaction with iodine—*i. e.*, should be free from amylo-dextrin (erythro-dextrin). Such dextrin is hard to procure.

†This and the next four should be added, after sterilization, by means of sterile pipettes. The ammonium salts may be obtained in a sterile condition without loss of ammonia by dissolving 10 grams in 200 cc. of water and forcing this through a Chamberland filter into a sterile flask, from which the proper quantity may be pipetted into the culture medium after sterilization.

‡Fig. 46.—Wooden carrier for fermentation-tubes, the flanging base being held under the grooves. Much reduced.

should be disturbed as little as possible after inoculation, and especially all tiltings or rough jarring should be avoided. They may be carried in a wooden rack (fig. 46). All culture-media, whether inoculated or not, should be protected from light.

Figs. 47, 48, 49 show fermentation-tubes in actual use.

The pattern of fermentation-tube preferred by the writer is that slight modification of Einhorn's tube designed by Dr. Theobald Smith (see Wilder Quarter Century Book). The tubes may be had from Emil Greiner, New York. Certain forms of tubes should not be used. One of these, a short, thick tube with a wide U, in use in some laboratories in this country, allows air to pass readily into the closed end and is entirely worthless. A sample tube of this sort was filled with

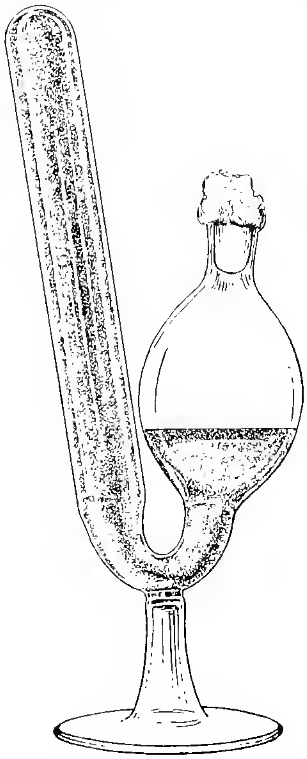
Fig. 47.*

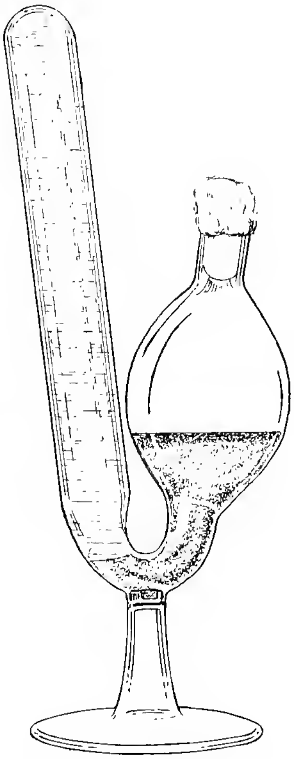
Fig. 48.†

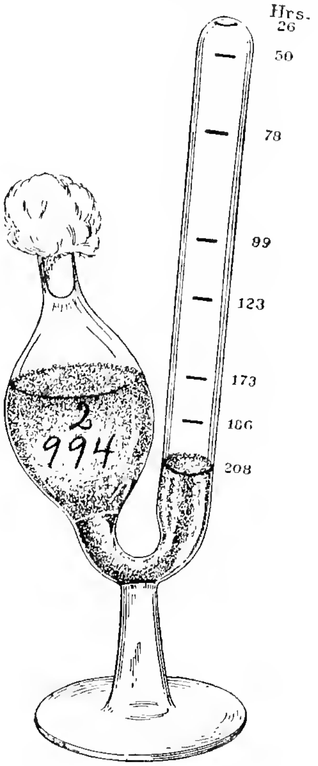

Fig. 49.‡

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*Fig. 47.—Fermentation-tube with *Bacillus tracheiphilus*, showing absence of gas and uniform clouding in open and closed end in the presence of grape-sugar. The fluid consisted of water, 400; Savory & Moore's peptone, 4; sodium chloride, 1; c. p. grape-sugar, 2; saturated solution carbonate of soda (20° C.), 20 drops, *i. e.*, enough to render the fluid slightly alkaline to litmus.

†Fig 48.—Fermentation-tube with *Bacillus tracheiphilus*, showing inability of organism to grow anaerobically with glycerin as the carbon food. Fluid, distilled water with 1 per cent Witte's peptonum siccum and 1 per cent Schering's c. p. glycerin. Copious growth in open end and in outer part of U; none in the closed end.

‡Fig. 49.—Fermentation-tube of cane-sugar peptone water inoculated with a white, gas-forming organism plated from a spot disease of sisal hemp. The total amount of gas produced and its rate of evolution at 20° to 23° C. are indicated by marks on the closed end of the tube.

beef-bouillon and steamed every twenty-four hours for seven or eight days, a large bubble being tilted out each time and appearing just as regularly during the next steaming. Naturally, no strict anaerobe would grow in such a tube and every aerobe would appear to be a facultative anaerobe. The neck of the fermentation-tube should be as narrow as consistent with filling and cleaning. All wide-necked tubes should be discarded. The behavior of the closed end with reference to the absorption of air may be tested by adding litmus-water and 5 per cent grape-sugar to the bouillon. On steaming, the litmus is reduced. If there is no air in the closed end the litmus remains reduced, while in the open end exposed to the air it soon oxidizes back to its original color.

Other things to be observed are:

(*3*) *Growth in hydrogen.*

(*4*) *Growth in carbon dioxide.*

(*5*) *Growth in vacuo, various degrees of exhaustion.*

(*6*) *Growth in vacuo, remnant of oxygen absorbed by the mixture of caustic potash and pyrogallol* (same as pyrogallic acid).

(*7*) *Growth in nitrogen* (air with the oxygen absorbed, normal air-pressure).

The hydrogen and carbon dioxide, which are required in considerable quantities, may be generated in Kipp gas-generators. There is a choice in generators. The writer has not found any kind which is entirely satisfactory. The one which has given the least trouble is shown in fig. 50. The objection to this generator is the large volume of dead acid which soon accumulates at the bottom. The accumulation of dead acid is entirely obviated in the de Koninck generator, but the writer has only recently obtained this apparatus and has not yet had enough experience with it to speak unqualifiedly. It furnishes a large amount of gas and its generation may be stopped very quickly, but the acid chamber is inconveniently bulky (10 liters) and in case of breakage a destructive flood would be poured out into the laboratory. To avoid this the apparatus should be set into a deep enameled iron pan. The action of the apparatus depends on the fact

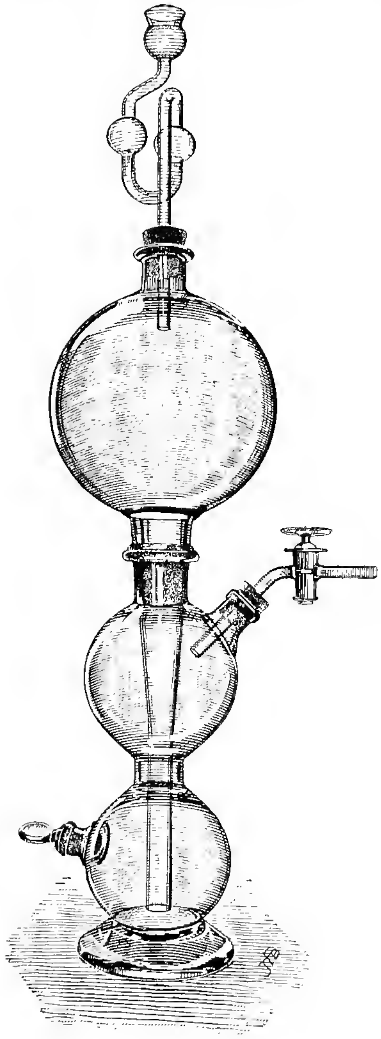

Fig. 50.*

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*Fig. 50.—Kipp gas-generator for making carbon dioxide or hydrogen. When not in use the pressure of the gas forces the acid off the marble or zinc (in the middle compartment) and stops its evolution. Much reduced.

that acid on which zinc has reacted has a greater specific gravity than unused acid and diffuses downward through the whole fluid when it is forced back from the zinc-chamber into the top of the acid-tank.

Another form of hydrogen generator is shown on plate 7. When in use the lower bulb is filled with acid and also the stem of the upper one. This gives a sufficient column of liquid to force the gas through the five wash-bottles. All the joints should be coated with Darwin's wax-mixture, set together firmly, and wired in place. Excessive liberation of hydrogen sulphide is avoided by standing the generator in ice water. The ruler is 12 inches long. The same style of apparatus may be used for the generation of carbon dioxide.

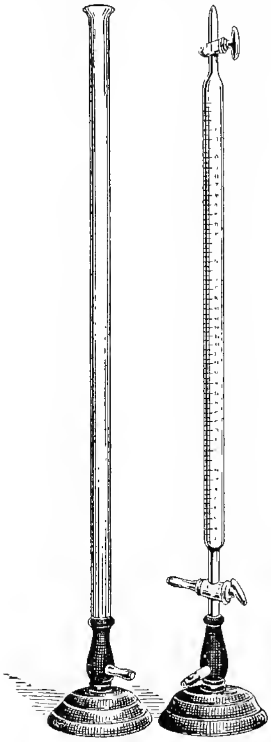

Fig. 51.*

These gases must, of course, be carefully washed to remove accidental poisonous impurities, by passing them through wash-bottles containing various solutions. For the carbon dioxide, which is usually generated from c.p. hydrochloric acid, diluted with twice its volume of boiled water, and marble chips (which should be boiled in advance), it is sufficient for many purposes to pass it through strong solutions of sodium hydrate (10 per cent), potassium permanganate (10 per cent), and water, arranged in the order indicated. Most of the oxygen may be removed by passing through three wash-bottles containing a mixture of pyrogallol and strong caustic-potash water or caustic-soda water (10 per cent). When in use the stopcock between the generator and the first wash-bottle must not be cut off, otherwise the small amount of carbon dioxide in the wash-bottle will soon be absorbed by the soda and fluids will be forced over (backward) from the other bottles by inequalities in the gas-pressure. The place to cut off the gas-flow is close to the Novy jar or other receptacle.

For testing the purity of the gas, *i. e.*, its freedom from air, 100 cc. may be drawn off into a Hempel burette (fig. 51), equalized with the air-pressure and run into the simple Hempel pipette for liquid reagents (fig. 52), the bulb of which is filled with strong potash water (2 water + 1 potassium hydroxide). If any gas remains after thorough exposure to the potash, it may be measured by passing it back into the burette. One should get with the pipette an *iron* stand and about 2 yards of capillary glass tubing.

The scrap-zinc used for generating the hydrogen should contain some lead, but should be free from arsenic, antimony, and phosphorus, and the sulphuric acid should be chemically pure. For use the acid is diluted largely with water (1 : 9). Hydrogen generated with zinc, especially if the evolution is rapid so that the solution is warmed, contains considerable hydrogen sulphide and may contain phosphureted hydrogen or arseniureted hydrogen; it should therefore be passed not too rapidly through the following solutions in the order indicated: Saturated solution of lead acetate, 5 per cent solution of silver nitrate, 10 per cent potassium permanganate, 10 per cent sodium hydrate containing pyrogallol, distilled water. When ready for use the purity of the hydrogen may be tested by burning in test-tubes (mouth down , and also, if necessary, by the ordinary methods of gas-analysis. To avoid the evolution of hydrogen sulphide the generator may be plunged into a jar of ice-water, as shown in plate 7. Special care must be taken in sealing jars containing hydrogen, otherwise it will escape. In use, the gas is allowed to bubble slowly through the fluids into the culture-chamber, a large well-clamped Novy jar, the other tubular opening of which is connected air-tight with the tube of the vacuum pipe. The jar is first pumped out and the hydrogen is then allowed to enter. When the jar is full, the glass stopcock nearest it (at the left in plate 7) is turned, and then, after allowing a few minutes for diffusion, the mixture of air and gas is pumped out. The vacuum cock is then turned off and the hydrogen is again turned on slowly. This process is repeated five or six times, the gas being passed into the jar very slowly the last two times, so that it may be washed very clean. The Novy jar is then sealed, disconnected, and set away in the dark.

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*Fig. 51.—Hempel's burettes for gas-analysis. Height, 25 inches.

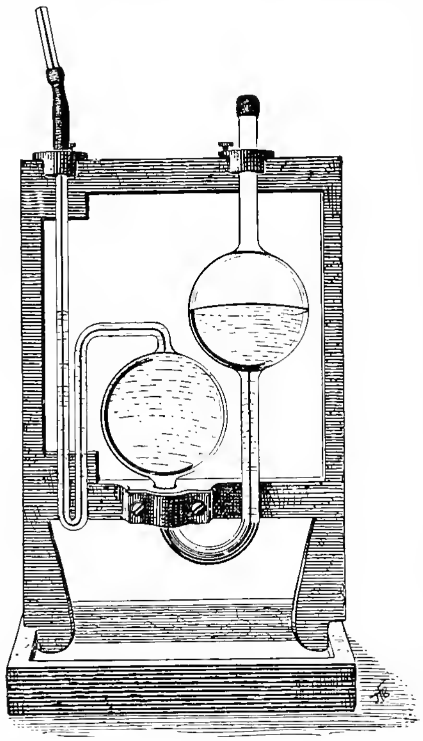

Fig. 52.*

The gas must, of course, enter each wash-bottle through the long stem. It is desirable to have each wash-bottle two-thirds full of fluid, and there must be no leaks in any part of the apparatus.† The hydrogen should be cut off before each exhaustion of the jar by turning the stop-cock nearest the jar. The cock also should be turned off before sealing glass tubes with flame and it must, of course, be known that the gas is free from admixture with air, otherwise an explosion will occur.

It is easier to keep air out of gases than to remove it. The greatest care should therefore be taken to drive it out of a culture medium before it is inoculated. For the same reason gas should be allowed to flow for some time before it is collected so as to displace air which may have diffused into the generator and wash-bottles. This is also the reason why the water which is used to dilute the acid and the marble chips should be boiled. If there is much air mixed with the gas it is not at all likely that a single wash-bottle of sodium hydroxide and pyrogallol,

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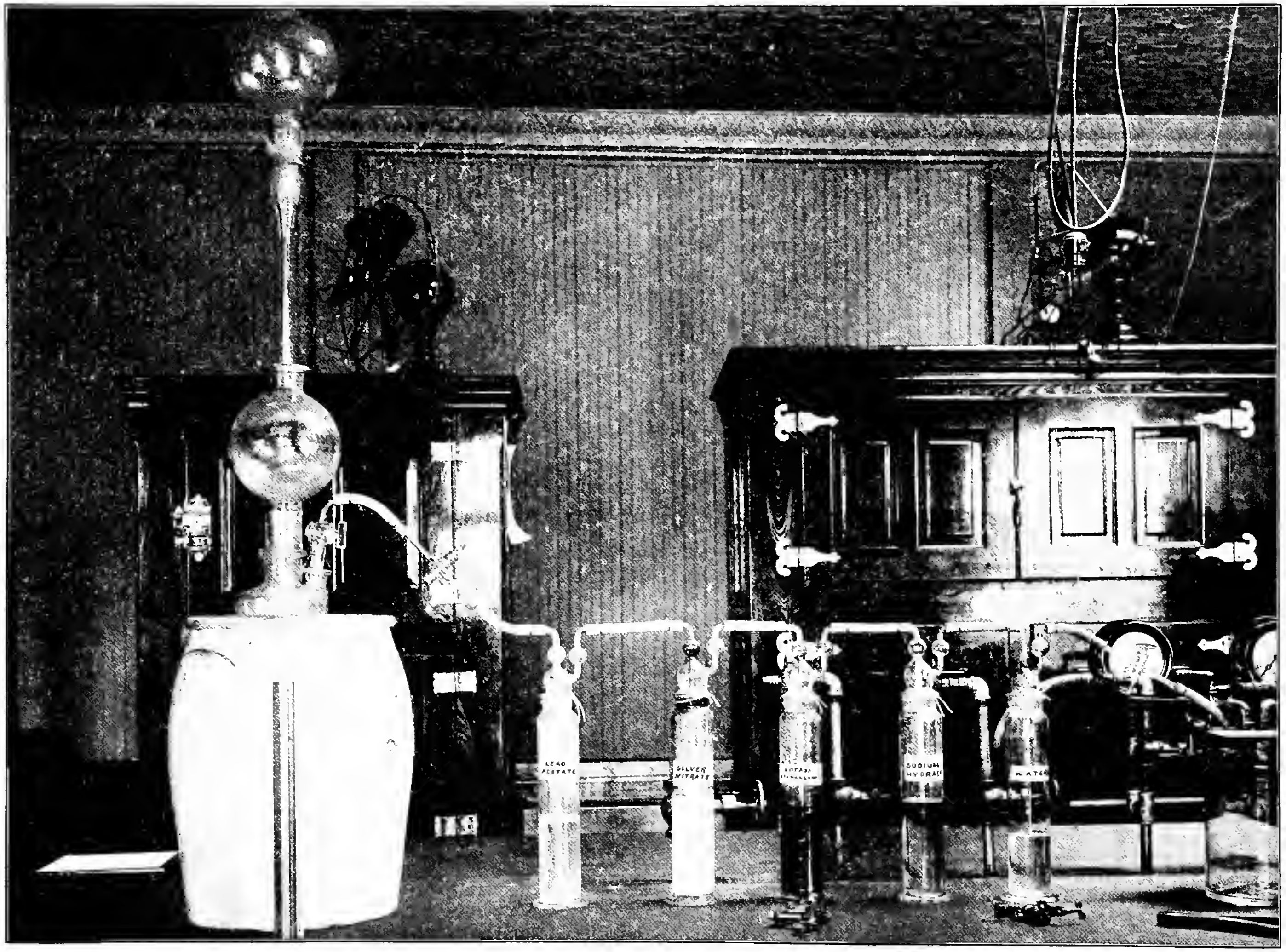
*Fig. 52.—Hempel's simple pipette for liquid reagents used in gas-analysis. Breadth of stand, 7 inches.

†Consult a paper by Ewell, Centralb. f. Bakt., 2 Abt., III Bd., p. 188.

PLATE 7.



Hydrogen generator with wash-bottles.

Back of the table are the cool boxes containing stock-cultures of various organisms; between the two is a gelatin box; at the extreme left is one corner of a large ice-box.

or even two or three in series, will completely remove it, since the bubbles of gas are in contact with the fluid only at their surface and for a very brief time. Hydrogen must be passed through 5 wash-bottles of sodium hydroxide and pyrogallol if every trace of oxygen is to be removed. From nitrogen or carbon dioxide the last traces of oxygen may be removed by passing it over copper filings inclosed in a piece of gas-pipe which is heated red hot in a small furnace containing about 20 Bunsen flames in series. The gas-pipe may be 0.75 inch in diameter and about 3 feet long, plugged at the ends with tight-fitting rubber stoppers, the middle 2 feet filled with the copper fragments. The gas should be allowed to flow only in rapid bubbles, not in a stream (Dr. Day).

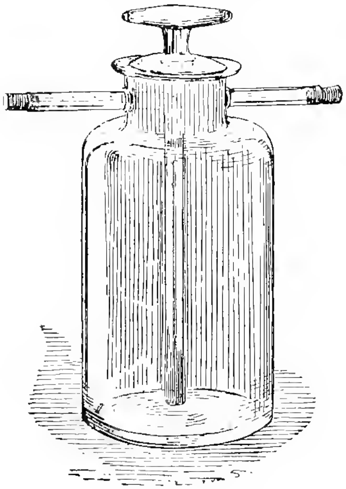


Fig. 53.*

The test-tube cultures may be placed in Novy jars, securely waxed (fig. 53), or in large, thick-walled test-tubes made impervious with sealing wax (see Sternberg, Manual, fig. 53; Text-Book, fig. 53). Media designed for use in any of these gases should be resteamed immediately before inoculation, and if one is experimenting with unknown or with very sensitive anaerobes the boiled media should be allowed to cool in an atmosphere of hydrogen. Francis Darwin's wax-mixture has been found useful for luting.

When large Novy jars are used (fig. 54), the thoroughly waxed gaskets must be clamped down securely and tested for leaks by preliminary exhaustions. If any are discovered, additional wax must be used and the clamps must be screwed tighter. To determine whether there is any subsequent entrance of air it is always best to include along with the cultures one or more tubes containing some substance which is reduced in the absence of free oxygen, but which readily oxidizes to some different color as soon as traces of air are mixed with the gas in the jar. Methylene blue in recently steamed bouillon or gelatin with 5 per cent grape-sugar is one of the best pigments for this purpose. In the absence of free oxygen it becomes a colorless substance; with the entrance of traces of air it becomes blue. Usually, however, the fluid or solid holds on to a trace of color at its surface. A solution of bilirubin is also said to be very sensitive to free oxygen and a good test for its presence.

Some care is necessary in order to avoid erroneous conclusions when pyrogallol and caustic potash are used to absorb the oxygen. The vessel must not leak, enough of the mixture must be used to absorb all the oxygen, and the action must be rapid enough so that the oxygen will have been removed completely before visible growth of the organism can possibly have taken place. Neglect of these precautions has led to the statement that certain strict aerobes are able to grow on ordinary media in the absence of oxygen, and that anaerobes are very uncertain in their behavior on standard media. Old pyrogallic acid should be avoided and some preliminary experiments should be made as to the rapidity of the absorption of the oxygen from a given space before the organism is tested. The writer found one brand of pyrogallol which removed the oxygen from a small space in six hours, another required about eighteen hours, a third required several days (time enough for a strictly aerobic organism to make a visible growth). Leaks may be detected readily by including with the cultures a fermentation-tube, the inclosed arm filled with water except for a small bubble of air. On absorption of the oxygen this bubble expands to a diameter which should remain constant if the jar continues air-tight.

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*Fig. 53.—Novy jar. Small size (wide mouth) for test-tube cultures. Only those with mouths at least 2½ inches wide are serviceable. Height to mouth of jar, 7¼ inches.

The gas remaining in receptacles from which the oxygen has been removed by the potash-pyrogallol method is not pure nitrogen, but nitrogen plus a variable small amount of carbon monoxide, which is said to be most abundant when the oxygen is absorbed slowly. This small amount of CO is harmless to many bacteria, but the writer has some reason for suspecting that it is injurious to others, even if it does not entirely inhibit growth.

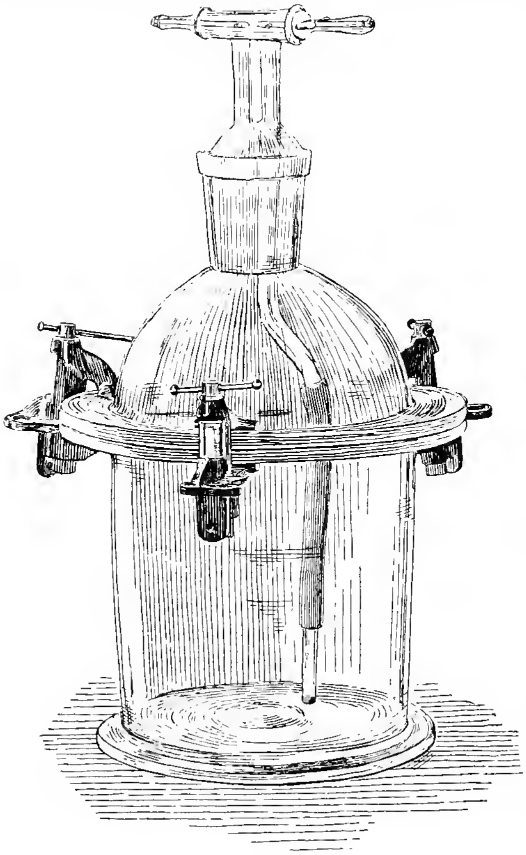

Fig. 54.*

The writer has found the following contrivance (fig. 55) a very simple one for testing the ability of organisms to grow in nitrogen: A U-tube of thick, clear glass, with arms about 10 to 12 inches long, open at the ends and having a uniform inside diameter of about 1 inch, serves as the culture-chamber and gas-receptacle. Two short, rimless, cotton-plugged test-tubes containing the media to be tested are inoculated and thrust one above the other into one arm of the U-tube, into which is then crowded a tight-fitting, soft, rubber stopper. This end is finally buried for an inch or so in a small beaker of glycerin and is perfectly air-tight. A rimless test-tube about 5 inches (13 cm.) long and of a diameter such that it will just slip easily up the other arm of the U-tube, is now packed by means of a pencil or glass rod with 8 or 10 grams of pyrogallic acid, covered quickly with 25 cc. of 10 or 15 per cent caustic-potash water, and slipped up the open end of the tube, which is immediately plunged into a dish of mercury and held there (under a shelf) until enough of the oxygen is absorbed so that it will stay down of its own weight. The exposure should be made at 25° or 30° C., or at least at temperatures considerably above zero, since the absorption of the oxygen is slow in cool air.

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*Fig. 54.—Novy jar of large size for Petri dishes and numerous test-tube cultures. Clamped as when in use. Between the clamped parts is a rubber gasket, carefully waxed and vaselined. Darwin's wax-mixture is advised. The writer also usually wires in the waxed top parts. The gas inflow is cut off by twisting the uppermost (horizontal) ground-glass stopper, which must be carefully vaselined. One-third actual size.

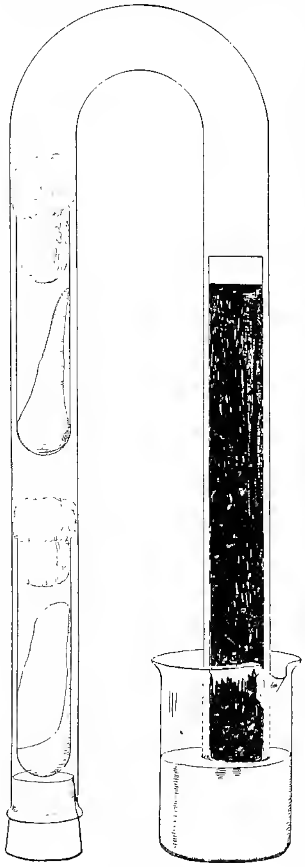

Fig. 55.*

The tube containing the pyrogallic acid and potash mixture floats on the mercury and rises, of course, in the arm of the U-tube as the oxygen is absorbed and the mercury enters it. This tube must not, therefore, be too long so as to hit against the curves of the U-tube before all of the oxygen has been absorbed; otherwise the mercury will pass up between the two tubes and overflow into the mixture. In other words, several centimeters must be allowed for the rise of the mercury.

A few experiments will determine how much of the mixture is necessary for a tube of a given bore and how long it takes to absorb all of the oxygen.† The level of the mercury in the open end with all the oxygen absorbed may be recorded by a scratch on the tube as a rough guide in subsequent work. At least half a dozen of these tubes will be found useful. They may be made in any laboratory or may be procured from dealers in glassware.

In the use of carbon dioxide, especially with sensitive organisms, two factors must be considered, (1) the simple exclusion of air, as in case of hydrogen, and (2) the change in the reaction of the medium due to absorption of the gas (formation of carbonic acid).

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*FIG. 55.—A simple device for growing organisms in air deprived of its oxygen. In the left arm are the cultures; in the right arm is a test-tube containing a mixture of pyrogallol and caustic-potash water. The beaker contains mercury. About one-third actual size. A modification of Ganong's apparatus for study of germinating seeds.

† Macé states that 1 gram of the pyrogallol and 10 cc. of the 10 per cent potash-water are sufficient for each 100 cc. of air space.

LUMINOSITY.

Numerous saprophytic bacteria are luminous under certain special conditions. Luminosity is also a striking characteristic of at least one bacterial animal disease—the white disease, or sluggish disease, of sand fleas (*Talorchestia longicornis* and *T. megalophthalmia*), common on the shores of France and of Massachusetts at Woods Hole. Decaying potatoes and other vegetables are sometimes luminous. The question of luminosity should therefore be kept in mind by the student of plant diseases, although no luminous species are known to live in plants. Most of these interesting luminous bacteria have been found in salt water or near it, or on the flesh of quadrupeds and fish. Gorham has been able to grow them on strictly synthetic media. The most recent treatise is by Hans Molisch (Leuchtende Pflanzen, Jena, Gustav Fischer, 1904, pp. IX, 169, with 2 plates and 16 text figures).

Molisch records 26 species of luminous bacteria. He found that salt-water fish and the flesh of cattle exposed in the markets were very often luminous—48 per cent of 70 samples of the latter and nearly all the former. Of horse flesh 65 per cent and of cattle flesh 89 per cent became luminous on putting it into 3 per cent solution of sodium chloride, allowing a part of it to project into the air. Fresh-water fish are very seldom luminous. Seedlings exposed to Petri-dish poured plates curved heliotropically toward the light, but they did not become green. Other chlorides than that of sodium stimulate growth and light-production, *e. g.*, potassium, magnesium, or calcium chloride. Certain non-chlorides, such as potassium iodide, potassium sulfate, and magnesium sulfate have the same action (3 per cent or less). Potassium nitrate was also active on *B. phosphoreum* but not on *B. photogenus*. Manganese sulfate stimulated growth very noticeably but had no corresponding effect on the luminosity, which was weak. The spectrum of *B. phosphoreum* differs from that of the West Indian beetle, *Pyrophorus noctilucus*, and from that of a luminous fungus known as mycelium X. No biological importance is attributed to the luminosity which is ascribed to an hypothetical *photogen*. It is an oxidization phenomenon which can take place only in the presence of free oxygen. A temperature of 30° C. for forty-eight hours is sufficient to kill *B. phosphoreum* in gelatin cultures. The minimum temperature for this organism is below zero, the optimum is about 16° to 18° C., and the maximum is 28° C. The bacteria are luminous from minus 5° to plus 28° C. Light production is most intense from 5° to 20° C.

FERMENTATION PRODUCTS.

The old conception of fermentation involves an evolution of gas (*fervere*, to boil), but the term is now used with a wider meaning. Like many other terms, it is difficult to use it always logically. In general, it means the breaking up of carbon compounds into simpler substances, either by the direct action of the protoplasm of the organism (hypothetical) or by chemical substances (enzyms, diastases) secreted by the protoplasm. Acids and alcohols are produced; gases may or may not be evolved. Other volatile products are also produced, *e. g.*, esters, but usually only in very small quantities. Certain of the bacterial fermentations are of large commercial importance, *e. g.*, the acetic, the lactic. The breaking up of albumen and other complex nitrogen compounds, *i. e.*, putrefaction, is also sometimes called fermentation, and at present there is really no very sharp line to be drawn. Consult Green and Duclaux for the English and French views (Bibliog., XX). The student should observe:

(1) Gases. Amount, rate of development, kinds (carbon dioxide, oxygen, hydrogen, nitrogen, marsh gas).

(2) Acids. Volatile and non-volatile (lactic, acetic, butyric, etc.).

(3) Alcohols (ethyl, methyl, butyl, glycerin, mannit, etc.).

(4) Ethers and esters.

(5) Aldehyds, sugars, gums.

(6) Albumoses, peptones, amido-bodies.

The isolation and determination of the amount of these various products belongs to the province of the chemist, but the work should be done in the bacteriological laboratory and under the eye of the biologist if all sorts of errors, due to the unsuspected multiplication of intruding organisms, are not to creep in and render the work worthless. Only some crude determinations, as of proportion of the various gases evolved, may be made by the bacteriologist who is not a chemist. The volume of gas evolved from day to day may be measured in fermentation-tubes (fig. 49). Frost has devised a convenient gasometer for roughly estimating it (see his Laboratory Guide, plate I). These may be made in any laboratory out of cardboard.

If the gas is carbon dioxide it may be absorbed by shaking with 10 per cent NaOH. To do this, fill the bowl (fig. 49) even full of the strong caustic-soda water, place the thumb or forefinger over the mouth so as not to include any air, invert the tube so that the gas shall flow into the bowl and come into contact with the alkali, and shake vigorously until all of the carbon dioxide is absorbed. Tilt the fluid back into the open end, and remove the finger so as to equalize the pressure. If any gas remains after equalizing the air-pressure, place the finger over the mouth of the tube, tilt the gas into the bowl and apply a lighted match close to the mouth as the finger is removed. If it is hydrogen or marsh gas it will explode in the open end of the tube when the finger is removed and a flame applied. If it is nitrogen it will not support combustion (see Bibliog., XX, especially '90 Smith and '93 Smith).

How distinguish marsh gas from hydrogen?

Organisms easily inhibited by their own acid products may be kept alive a much longer time by adding a little calcium carbonate to the bouillon or agar.

In simple tests for acids, discard bright blue litmus paper, which is very sensitive to carbonic acid (try carbonated water on it), and use instead a good grade of reddish-violet (neutral) litmus paper. Such paper may be made in the laboratory (the best way) or may be purchased of H. Struers, Copenhagen.

ALKALIES (AMMONIA, AMINS, CARBONATES OF THE ALKALI METALS).

Determine rapidity of formation. Note that they are often masked by the simultaneous formation of acids. Try the litmus test and Nessler's test. Do not put Nessler's solution into the culture fluid, but expose it to steam from the culture. Observe the behavior of the organism when grown in peptone rosolic-acid water with just enough HCl added to counteract the alkali in the peptone, and in neutral or slightly acid peptone-water or sugar-free bouillon containing acid fuchsin. On titration of acids and alkalies see Sutton (Bibliography of General Literature, IV).

REDUCING POWERS.

Determine rapidity of reduction of litmus, methylene blue, and indigo carmine in various fluids and solids (with and without grape-sugar). Probably all bacteria can reduce litmus, etc., but as the rapidity of reduction varies greatly in different species and in different media, it is desirable to make comparative tests. Consult a recent paper by Albert Maassen ("Ueber das Reduktionsvermögen der Bakterien, und ueber reduzierende Stoffe in pflanzlichen und tierischen Zellen," Arb. a. d. Kais. Gesundheitsamte, Bd. XXI, 3 Heft, 1904, pp. 377-384).

HYDROGEN SULPHIDE.

This gas is the product of a reduction. From what media and under what conditions is hydrogen sulphide given off with browning of lead acetate paper? This paper is readily prepared by dipping strips of white filter paper into a strong solution of lead acetate in distilled water. It should be kept in a tight tin box or a glass-stoppered bottle. Probably most, if not all, bacteria are able to produce hydrogen sulphide in nutrient media containing readily decomposable sulphur compounds. Is an enzyme necessary? When an organism grays potato cylinders in test-tubes, why is no hydrogen sulphide given off? The student should read papers by Petri and Maassen (Bibliog., XXVIII).

MERCAPTAN AND OTHER ODORS.

We need an odor chart to go along with our color charts. If we could have a set of standard substances with peculiar smells for comparison with the many odors evolved from bacterial cultures it would certainly be a great convenience. The difficulty at present is that the judgment of people varies greatly, in many instances, as to what the smell should be likened. As it is, the bacteriologist must do the best he can to define these penetrating smells, which are sometimes very characteristic of particular organisms. Some of the fishy odors are due to amins. Mercaptan is a very vile-smelling sulphur compound.

INDOL, PHENOL, LEUCIN, TYROSIN, ETC.

The production of indol is best studied in peptonized beef-broth naturally free from sugar or which has been deprived of its muscle sugar by growing in it (for a few hours only) *Bacillus coli* (Theobald Smith), after which it should be filtered clear. If *B. coli* or *B. cloacae* will not produce gas in beef-broth in the closed end of fermentation-tubes, it is free from sugar and suitable for this use. Many organisms give the indol reaction in Uschinsky's solution to which peptone has been added. The writer has never been able to obtain the indol reaction in any culture medium which did not contain peptone (using this word in the commercial sense.) Cultures which do not show the red reaction with sodium nitrite (0.02 per cent solution) and sulphuric acid at room temperature will frequently do so when put into hot water for five minutes (70° to 80° C.). The browning of media due to excess of sodium nitrite must not be mistaken for this pink or red reaction. Uninoculated tubes should be included in the test, which may be made on the second and tenth day.

For methods of determining phenol see Lewandowski in Deutsche Med. Wochenschrift, 1890, p. 1186, and Chester's Manual, p. 33. Schmidt (Bd. II, p. 1008) gives the following as a qualitative reaction for tyrosin: Dissolve by boiling in water and add a solution of mercuric nitrate. The red reaction is sharper if a little fuming nitric acid diluted in water is added. Try also the violet reaction with neutral iron chloride.[*] Leucin crystallizes in white soft scales.

REDUCTION OF NITRATES (AND MORE COMPLEX NITROGEN COMPOUNDS) TO NITRITES, TO AMMONIA, AND TO FREE NITROGEN.

For the pathologist the iodine-starch reaction is the most satisfactory test for nitrites, because it is not superlatively sensitive and consequently does not indicate traces of nitrite absorbed from the air. It is made as follows: Twenty-five cubic centimeters of distilled water are added to one-half gram (more or less) of pure potato starch and the fluid boiled. One cubic centimeter or more of this starch-water and 1 cc. of *freshly prepared* potassium-iodide water (1 : 250) are now put into the culture fluid, to which is then added a few drops of strong sulphuric-acid water (2 : 1). If any appreciable quantity of nitrite is present the culture immediately becomes blue-black from the liberation of free iodine, which acts upon the starch. Old potassium iodide water should never be used without first testing carefully, as it usually contains some *free iodine*. It is always best to first make a trial test without the bacteria. Commercial starch frequently contains products of bacterial decomposition and starch prepared aseptically should be substituted.

At least one-third of the organisms which have fallen under the writer's observation in recent years give the nitrite reaction when grown in peptonized beef-bouillon containing potassium nitrate.

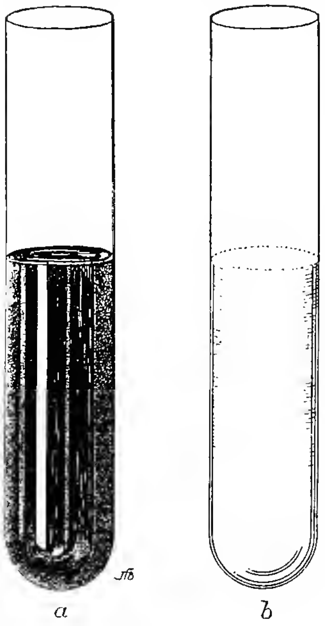

Fig. 56.[†]

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[*] Mann (p. 323) gives the following as a specific tyrosin reaction: Denigès has recommended the well-known phenol aldehyde reaction for the detection of tyrosin. Nasse, in repeating Denigès' observations, has found the following to be a very delicate test for tyrosin, as neither proteids nor peptones give the color-reaction. Proceed thus: Add a few drops of formol solution to concentrated sulphuric acid, when, on warming with tyrosin, a brown-red color is obtained, which, on addition of acetic acid, becomes green.

[†] FIG. 56.—*Bacterium syringae* (van Hall). Nitrate bouillon cultures 5 days old, to each of which has been added boiled starch water, potassium iodide water, and sulphuric acid. In tube *a* the potassium nitrate was reduced to the nitrite, and on addition of the reagents free iodine was liberated, and the starch blued. In the other no nitrite had formed, no iodine was liberated, and the starch remained colorless. For discrepancy see text.

Fig. 56 shows how differently quite similar-looking cultures may react when submitted to this test. Both of these organisms were received from van Hall under the name of *Pseudomonas syringæ*, *a* being van Hall's own isolation and *b* being supposedly a subculture from Beyerinck's isolation. Neither one would produce any blight in lilac shoots.

There is no simple way known to the writer of distinguishing ammonia from the amins, as both react to Nessler's reagent. Nitrogen may be distinguished from the other gases of fermentation by the fact that it is not absorbed by sodium or potassium hydroxide and will not burn or support combustion. This gas is produced readily from nitrates by a number of green-fluorescent organisms (dung-destroyers) but not by all of them.

FIXATION OF FREE NITROGEN AND THE OXIDATION OF AMMONIA AND AMMONIUM SALTS TO NITRITES AND NITRATES.

These processes are probably common enough to organisms of the soil, many of which have not been investigated, but they are not known to be brought about by plant parasites exclusive of the root-tubercle bacilli of the Leguminosae, which some believe to be parasites (see Peirce).* They are believed to be of rare occurrence in bacteria which grow well on ordinary culture media.

The nodules on roots of plants will hereafter be considered more fully. The reader should consult a paper by Geo. T. Moore on "Soil Inoculation for Legumes," Bureau of Plant Industry, United States Department of Agriculture, Bull. 71, January 23, 1905; and one by Maria Dawson, "Further Observations on the Nature and Functions of the Nodules of Leguminous Plants," Philosophical Transactions Royal Society of London, Series B, Vol. CXCIII, pp. 51-67, 1900, with 2 plates.

ASSIMILATION OF CARBON DIOXIDE.

Some soil organisms are believed to obtain their carbon directly from carbon dioxide, and would thus be exceptions to the law that all non-chlorophyllous plants must obtain their carbon from organic substances. This supposition, while probably true, has not, we believe, been established satisfactorily. Its elucidation offers a most interesting line of research (see Bibliog., XXVI.)

PIGMENTS.

Bacterial growths are often bright colored, and an examination of the pigments should form part of one's study of an organism. They may be considered as follows:

(1) Under what conditions formed? Can they be eliminated by growing the organisms in the dark or under unfavorable conditions, *e. g.*, near the maximum or minimum temperature? *Bacillus prodigiosus* is a favorable organism for experiment.

(2) In what soluble (water, hydrogen-peroxide in water, ethyl alcohol, methyl alcohol, glycerin, acetic ether, petroleum ether, sulphuric ether, acetone, chloroform, turpentine, benzine, benzole, xylol, toluol, carbon bisulphide, etc.)? The pigment should be tested in as many solvents as possible.

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*Peirce, George James. The Root-tubercles of Bur Clover (*Medicago denticulata* Willd.) and of Some Other Leguminous Plants. Proc. Calif. Acad. Sci., 3d series, Botany, Vol. II, No. 10, San Francisco, Cal., June 21, 1902, pp. 295-328, with 1 plate.

(3) How are they acted on by acids, alkalies, and other reagents?

(4) Of what use are they to the organism? Are they oxidation-products? Examine spectroscopically, if possible.

On the addition of acids or alkalies, a bacterial pigment may remain unchanged, may be changed into some different color, may be destroyed, or may be converted into some colorless compound which will regain its original color on changing back the reaction. The yellow pigment of several species of *Bacterium* (*Pseudomonas*) remains unchanged in the presence of acids and alkalies. The blood-red color of *Bacillus prodigiosus* becomes carmine in the presence of certain acids and yellowish-brown in the presence of certain alkalies. The blue color of *Bacterium syncyaneum* is said to be produced only in acid milk. The beautiful green fluorescence of *Bacterium pericarditidis* (*Bacillus pyocyaneus pericarditidis*), and probably of all this group of bacteria, is produced only in alkaline media. According to Jordan two pigments are normally produced by many green-fluorescent organisms. The blue pigment pyocyanin is visible by gaslight and is soluble in chloroform. The green-fluorescent pigment is insoluble in chloroform and yellowish by gaslight. By this latter test the two can be distinguished when mixed. Soluble phosphates and sulphates are necessary for the production of green fluorescence. The ability to produce pyocyanin is easily lost. Its production in the culture-medium, unlike that of the fluorescine, is not dependent on the presence of phosphates or sulfates. Pyocyanin turns red with acids, fluorescine becomes colorless; both return to their original color on adding alkali sufficient to change the reaction. "Asparagin, ammonium succinate, ammonium lactate, and ammonium citrate all proved suitable for the development of the fluorescent pigment." The yellow and black pigments are the result of oxidations. (See papers by Gessard, Thumm, and Jordan, Bibliog., XXIII).

The pigments of bacteria range from one end of the spectrum to the other. Thus we have various shades of black, brown, violet, indigo, blue, green, yellow, orange, and red. Many bacteria produce no pigment, *i. e.*, are white when seen in mass. Others produce several distinct pigments. Many of the plant parasites are yellow, *e. g.*, *Bacterium campestre*, *Bact. phaseoli*, *Bact. hyacinthi*, *Bact. Stewarti*, *Bact. juglandis.* Some of these yellow organisms stain the host-plant and certain nutrient substrata a deep brown. Other plant parasites are white but also stain the host and certain substrata brown, *e.g*, *Bacterium solanacearum*, *Bacillus carotovorus*, *B. aroideæ.* Others are pure white and are apparently destitute of any pigment-producing powers, *e.g.*, *Bacillus amylovorus*, *B. tracheiphilus.* Very many bacteria when grown on cooked potato produce a gray stain in this substratum, especially in that part freely exposed to the air, *i. e.*, out of the water.

Some other color changes in the host should be mentioned. Various brown and red stains visible in certain plants when attacked by bacteria are not attributable directly to the presence of the microorganisms in the tissues. These are oxidation phenomena likely to occur when the plants are wounded or destroyed by any agent whatsoever. A few illustrations will make my meaning clear. When the limbs of pear trees are destroyed by blight the foliage becomes black, but this blackening also occurs frequently when the flowers, green fruits, or foliage are killed by other causes. In the leaves of *Amaryllis atamasco* the writer obtained red stripes by injecting the yellow *Bacterium hyacinthi*, but no bacterial disease followed, and the same plant reddens when bruised. Broomcorn shows conspicuous red blotches when attacked by the broomcorn organism, but the parasite itself does not produce a red pigment, while the plant reddens easily as the result of aphis-punctures or wounds of any sort. Sugar-cane attacked by *Bacterium vascularum* shows a conspicuous red stain in the bundles, but other causes, such as the gnawings of an insect or the presence of a fungus, may lead to a similar stain, while the bacterium itself does not produce any red pigment.

CRYSTALS.

Determine the nature of the crystals observed in the various media. Many of these are double ammonium salts; others result from the action of trypsin on proteids. Crystals which are not due to the drying out of the media are common phenomena in old cultures of many sorts, especially if the media were not originally saturated with alkali (soda or potash). Fig. 57 shows two types of crystals formed in +15 nutrient agar by two green-fluorescent organisms received from van Hall as *Pseudomonas syringæ*, and a third type produced by the olive tubercle organism.

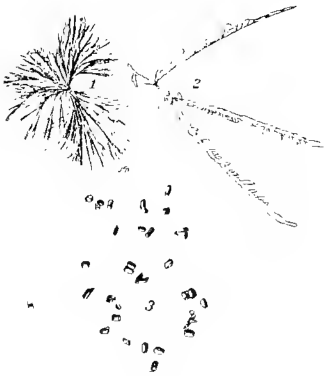

Fig. 57.*

QUESTION OF EXISTENCE OF ENZYMES.

The enzymes of English writers are the diastases of Duclaux. They are chemical substances, the exact composition of which has not been determined. They may be regarded as the working tools of protoplasm. The following are some of the best known kinds:

(1.) Diastasic (starch-destroying).
(2.) Inverting (sugar-splitting).
(3.) Cytohydrolytic (cellulose-dissolving.)
(4.) Proteolytic (peptonizing).
(5.) Lab or rennet (casein-forming).
(6.) Lipase (fat-splitting).
(7.) Pectic (pectin-splitting).
(8.) Oxidases (oxidizing).

Trypsin is common. Pepsin is not known to be produced by bacteria and should be searched for.

Many bacteria invert cane-sugar, but invertase is believed to be rare. This, however, may be an ill-founded conclusion. The experiments of various animal physiologists have shown that when cane-sugar is injected into the blood-stream it is excreted unchanged, and according to Julius Sachs cane-sugar, inulin, etc., must

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*Fig. 57.—Crystals formed in cultures of *Bacterium syringae* (van Hall). 1. From tube II, Aug. 14 (agar stock 693), from van Hall's II, *i. e.*, his own isolation corresponding to *a*, fig. 56. 2. From tube I, Aug. 14 (stock 693), from van Hall's I, which is from Beyerinck's old isolation (see *b*, fig. 56) × 3. Nos. 1 and 2 drawn Aug. 30, 1902. 3. Crystals formed on slant litmus-lactose agar which was inoculated with the organism causing olive-knot. About one-half inch of slant in middle part of culture 1 month old, *i. e.*, made January 20, 1904; drawn February 17-19. × 3. Temperature during growth, 20° to 25° C.

first be reduced to glucose (grape-sugar), before they can be used as food by plants. When no invertase has been detected the general hypothesis has been that this inversion was due to the direct action of the protoplasm, but the recent isolation by Buchner and others of an invertase (Zymase) from yeast, in which it was long believed that none existed, once more emphasizes the uncertainty of negative conclusions.

Diastase is common. Is there more than one kind, *i. e.*, a sort which can only convert the starch into amylodextrin and another which converts it into maltose and dextrine? In many cases, when the organism is grown on potato, the conversion is carried only a little way and stops, there being always a copious purple or red-purple reaction with iodine. In other cases, *e. g.*, when *Bacterium campestre* is grown on potato, the starch conversion is so complete that after a few weeks there is little or no color reaction when the potato-cylinder is mashed up and iodine water added. What makes this difference?

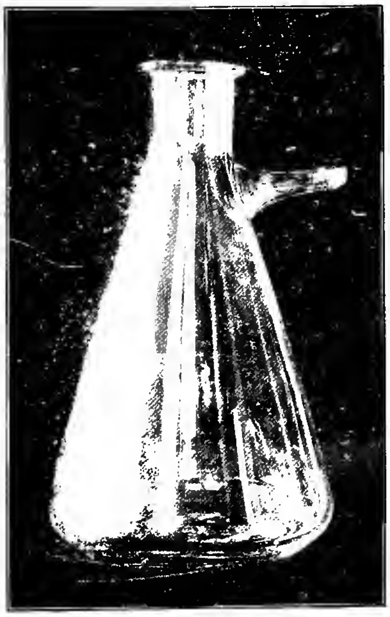

Fig. 58.*

A substance capable of dissolving the middle lamella appears to be common to all bacterial plant parasites and a true cytase presumably occurs, but much additional study is necessary. Probably several enzymes are confused under this name, just as several chemically different substances are still called "cellulose." The substance which dissolves the middle lamella in some cases is probably ammonium oxalate. The writer has not been able to dissolve it by means of pure oxalic acid, but that of turnips softens in ammonium oxalate.

The lab or rennet ferment is rather common. Its action should not be confused with the curdling of milk due to the formation of acids. Tests may be made in litmus milk. Is there more than one kind of such ferment? Some organisms coagulate the milk promptly into a solid mass which finally shrinks, extruding whey. Others cause the casein to separate out of the fluid very slowly as a multitude of separate particles which only become compacted very slowly.

The writer has not met with the oxidizing enzymes, unless the substance in bacterial cultures which causes rapid evolution of oxygen from hydrogen peroxide is such an enzyme, as Dr. Loew maintains (Bibliog., XLV). Many other enzymes undoubtedly occur and play their part. The student should search for emulsin, lipase, lactase, maltase (glucase), etc.

All known enzymes when freely exposed to steam heat are destroyed at temperatures considerably under 100° C. They are less sensitive to heat than the bacteria themselves, but are destroyed by a few minutes exposure to temperatures 15° to 30° C. (moist heat) above the thermal death-point of the organisms which have produced

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*Fig. 58.—Thick-walled Kitasato flask for filtration or evaporation *in vacuo*, etc. Much reduced.

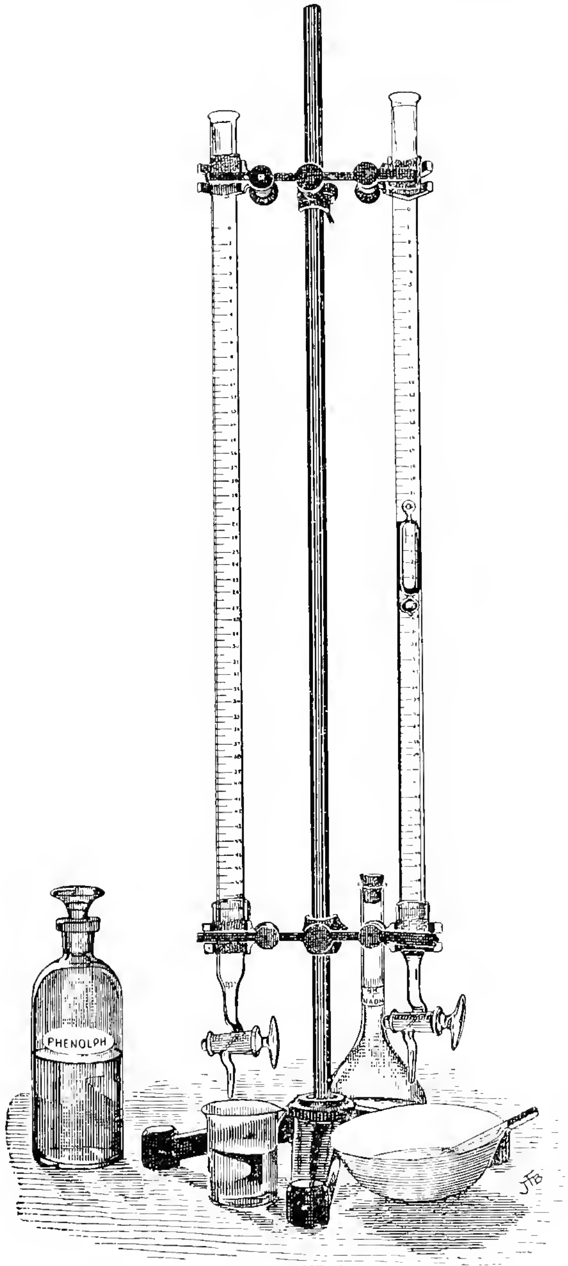

Fig. 59.[†]

them.[*] Some of them are very sensitive to the presence of acids, alkalies, strong alcohol, or antiseptics, or their action is inhibited by the presence of other enzymes or of products of enzymic fermentation in excess, or by the absence of some combining substance, such as lime or some weak acid. Some do not pass readily through the Chamberland filter or through filter papers. Some are destroyed at lower temperatures after precipitation. Some are not produced except in presence of the substance which they can decompose, but this is not true of all. Usually an organism produces more than one ferment and some bacteria are known to produce five or six. *Bacterium campestre* produces at least three and probably four, viz, diastasic, cytohydrolytic, proteolytic, and rennet. It also inverts cane-sugar, but it is not yet known whether this change is accomplished by means of an invertase. On enzymes derived from bacterial soft-rot organisms the reader should consult recent papers by Jones (Centralb. f. Bakt, 2 Abt., and Vermont Exp. Sta. Rep.). Levy has published an interesting paper on "Some physical properties of enzymes" (The Jour. Infect. Diseases, Vol. II, 1905, pp. 1-48).

For concentrating fluids in vacuo at low temperatures (50° to 60° C.) the thick-walled Kitasato flask shown in fig. 58 is very convenient. The side tube is attached to the suction-pipe of an air-pump and into the neck is thrust a rubber stopper carrying a thermometer and a U-shaped glass tube of small bore, the outer arm (36 inches long) ending in a beaker of mercury. Heat may be applied by means of a water-bath. By substituting a funnel for the thermometer the same device may be used to hasten the filtration of thick liquids, hard-pointed filter papers being employed.

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[*] The same amount of dry heat does not affect them, and Loeffler has recently advised exposure of thoroughly air-dried tissues and cultures to 150° C., dry heat, as an easy way of eliminating the bacteria prior to grinding and extraction of the uninjured enzymes and other soluble products. Non-sporiferous bacteria may be heated at 120° C. for 2 to 3 hours. Tissues and sporiferous bacteria should be heated at 150° C. for one-half hour. (Deutsche Med. Wochenschrift, Dec. 22, 1904.)

[†] Fig. 59.—Burettes used by the writer for titrating culture media. Twentieth-normal sodium hydrate is used to determine the acidity, and the medium is finally brought to the desired alkalinity with quadruple-normal sodium hydrate. The fluid is boiled and titrated hot, using phenolphthalein as the indicator. The burettes should be graduated to tenths of a cubic centimeter and should hold 50 cc. Alkali should not be allowed to stand in them.

SENSITIVENESS TO PLANT ACIDS.

The tests should be made with malic, citric, lactic, oxalic, and tartaric acids added to neutral beef-broth, peptone-water, or plant-broths, or to synthetic media (see Am. Nat., 1899, p. 208). It is best to titrate with $\frac{N}{10}$ or $\frac{N}{20}$ solutions, to acidify with $\frac{2N}{1}$ or $\frac{4N}{1}$ solutions, and to reckon the acidity in cubic centimeters of normal solution ($\frac{N}{1}$) required per liter of medium. If preferred, it may be calculated on 100 cc. portions and expressed in per cents, but there is no advantage in this, and it has the disadvantage of introducing fractions.

SENSITIVENESS TO ALKALIES (POTASSIUM OR SODIUM HYDRATE).

Determine in each case the optimum reaction of the medium for growth. For the majority of bacteria this is said to lie between +10 and +15 of Fuller's scale.† The best neutral litmus paper should be used freely, but acid and alkaline media should be titrated with phenolphthalein and $\frac{N}{10}$ or $\frac{N}{20}$ solutions. In some media—*e. g.*, gelatin, juices of various plants—the end-reaction with phenolphthalein and caustic soda is not very sharp. In these cases the titration should be stopped at the first trace of change of color. If one adds alkali until the fluid is decidedly red, then a distinct statement to that effect should be made, since otherwise no comparisons of any value can be made. All of the writer's + and — references to media are based on a reaction stopped at the first distinct trace of pink color. As much again alkali must sometimes be added to obtain a deep-red color.

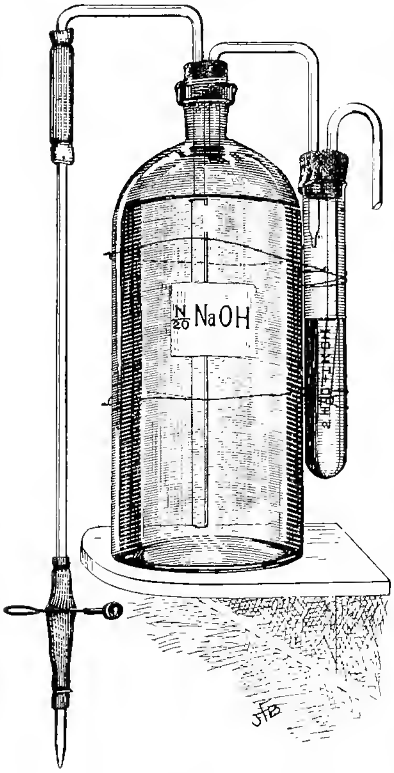

Fig. 60.*

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*Fig. 60.—Stock bottle of $\frac{N}{20}$ sodium hydrate solution. The small bottle at the right holds concentrated potash liquor to remove the carbon dioxide from the air which enters the bottle. About one-fourth actual size.

†The plus and minus on Fuller's scale denotes, respectively, acid and alkaline media. The +10, for example, means that exactly 10 cubic centimeters of normal alkali must be added to a liter of the culture medium to render it exactly neutral to phenolphthalein, and, correspondingly, —10 means that the fluid is alkaline to phenolphthalein and that 10 cc. of normal acid would need to be added to bring 1 liter back to the neutral point. The student should not confuse the litmus neutral point and the phenolphthalein neutral point, as they are about 23° apart, *e. g.*, +10 of Fuller's scale (acid side) is distinctly alkaline to litmus. (Consult '95, Fuller, Bibliog., XVI.)

The writer has used the foregoing method of determining the reaction of culture media for several years and has, in general, found it exceedingly exact and valuable, but it does not appear to be well adapted for determining the amount of alkali (ammonia and amins) produced by bacteria in culture media (see Sutton, Bibliog., IV). The apparatus required to make these titrations is shown in figs. 59 and 60.

Some experiments recently made by the writer with *Bacillus tracheiphilus* in peptonized beef-bouillons of varying degrees of acidity (acid of beef-juice) and alkalinity seem to show that toleration of sodium hydrate can be considerably increased by inoculating each time from alkaline bouillons rather than from acid ones. Taken from + 20 bouillon (descended from + 20 bouillon) this organism would cloud the same bouillon only down to 0; taken from 0 or —5 bouillons (descended from —2.7 bouillon) it would cloud the same bouillon down to —10 and probably farther, but not to —20. Bouillon containing various amounts of c. p. sodium chloride behaved in the same way. The organism would tolerate the largest amount of salt (1.5 to 2 per cent) when first grown in an alkaline bouillon. When inoculated from a + 20 bouillon the organism finally grew in 1 per cent salt bouillon, but only after a decided retardation, and would not grow at all in + 15 peptonized beef-bouillon containing 1.5 per cent sodium chloride.

Bacteria vary greatly in their toleration of acids and alkalies, the range of growth being from *minus* 100 (or more) of Fuller's scale to *plus* 100 (or more). The limits of growth are not known, but it is probable that the extremes of toleration in particular aberrant species is much greater than that here given, *e. g.*, on the acid side in sulphuric acid and vinegar bacteria, and on the alkaline side in case of those organisms which are able to grow in the lime-vats of tanning establishments and in alkaline springs. Lehmann & Neumann ('96, Bibliog., III), state that they have found bacteria that will endure 100 cc. of normal acid per liter of fluid culture media, *i. e.*, equal to about 1 per cent sulphuric acid. Some species are indifferent to a considerable degree, having a wide range of growth either side of the (phenolphthalein) neutral line; others prefer alkaline media; others acid media. Many are extremely sensitive to their own acid products (acetic, lactic, butyric, etc., acids). Not a few are differently affected by different acids and alkalies. Every new organism presents a whole series of special problems.

EFFECT OF DESICCATION.

Drops of fluid cultures or small masses of gelatin or agar cultures are spread on small (¼-inch) clean, sterile cover-glasses, in covered sterile Petri dishes, and are set away in the dark, in dry air (a dry room). The test is finally made by seizing one of these covers with a pair of sterile forceps and dropping it into a tube of sterile bouillon or other medium of a stock previously determined to be exactly adapted to the growth of the organism, *i. e.*, one which does not exert upon it any retarding influence. Occasionally a tube will become contaminated, but enough must be inoculated so that this will not affect the final result (20 at one time is not too many). Fluid cultures are preferred. Solid cultures do not give strictly comparable results.

Organisms believed to be non-sporiferous show great differences, some being killed by an exposure of a few minutes or a few hours, while others remain alive for many weeks. For further information see the special chapters on *Bacillus tracheiphilus*, *B. carotovorus*, *Bact. hyacinthi*, etc. Tests may also be made in air dried over sulfuric acid or calcium chloride. Harding & Prucha have shown recently that *Bacterium campestre* remains alive much longer when dried on cabbage seed than when dried on glass cover-slips. In their experiments this organism was dead on glass at the end of ten days, but alive on seed at the end of thirteen months.

EFFECT OF DIRECT SUNLIGHT.

The exposures should be made in a thin stratum of nutrient agar, not sowed too thickly (there may be several hundred colonies on the plate, if properly distributed), in thin-bottomed Petri dishes, to an unclouded sun for 5, 10, 15, 30, 45, and 60 minutes, a portion of the bottom of the plate, which is placed uppermost, being covered by some substance impervious to light, such as several folds of Manila paper or of the black paper which comes wrapped around photographic dry plates, covered in turn by white paper. Exposures of several hours are not recommended. If the layer of agar is very deep, or if the sowings are too thick, some organisms will screen others and all will not be killed. Ten cubic centimeters is a proper amount of agar to use for a plate having an area of 60 square centimeters. The latitude, altitude, time of year, time of day, and intensity of the light should also be recorded. In the summertime it is very important that the exposures should be made on blocks of ice or,

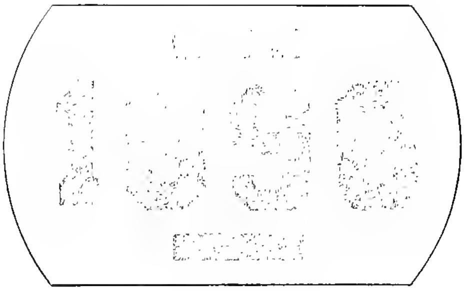

Fig. 61.*

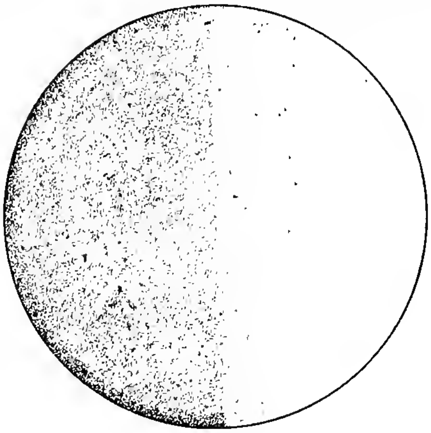

Fig. 62.†

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*Fig. 61.—Gelatin culture of *Bacillus amylovorus* (Burrill) Trev. in a Petri dish. Exposed in 1896 to direct sunlight for four hours on ice after covering portions of the plate with pasteboard figures. The bacteria grew only under the protected parts. Drawn from a photograph made after five days incubation of the culture at about 24° C. The temperature of the gelatin during exposure was about 25° C. Three-fifths natural size.

†Fig. 62.—Agar culture of *Bacterium phaseoli* (Erw. Sm.) in a Petri dish. Right one-half exposed to direct sunlight for thirty minutes, on ice, the other half protected by several folds of Manila paper. Dish then set away in the dark for several days. One-half natural size. The scattering colonies on the right side undoubtedly grew from bacteria which were sheltered from the direct rays of the sun by overlying organisms, *i. e.*, the plate was sown too thickly.

better, on larger Petri dishes filled with pounded ice; otherwise, in case of 30 to 60 minute exposures, the temperature may rise nearly or quite to that of the thermal death-point of the organism, and then we shall have the effect of heat complicating that of light. To avoid errors it is always best to take one-half of each dish as a check (rather than the whole of a separate dish), and the rise of temperature should be carefully recorded. In some tests made by the writer in Washington in May the temperature of the plates exposed in the open air to the sun for 45 minutes (without ice) rose from 25° to 51° C. Figs. 61 and 62 show the effect of sunlight upon thin sowings of *Bacillus amylovorus* and *Bacterium phaseoli* in poured-plate (Petri-dish) cultures.

VITALITY ON VARIOUS MEDIA.

By this I mean the determination of the resistance of organisms to their own decomposition products. This varies greatly. Much may be learned by the study of old cultures. Do not discard test-tube cultures until after many weeks. Examine frequently. Make transfers from tubes which have been inoculated for a year or more. Determine whether this vitality is due to spores or persists in the ordinary vegetative rods. On what kinds of media does a particular organism live longest? Can length of life be increased by occasionally neutralizing decomposition products (acids) with sterile carbonate of lime? or by occasional additions of food? Some bacteria are veritable revelers in filth; others are extremely sensitive; all are soon under abnormal conditions in our culture-tubes.

Another way of keeping bacteria alive for a long time is by reducing their growth to a minimum. Stock-cultures, especially of perishable organisms, should, generally speaking, be kept in the ice-box at temperatures under 15° C. This greatly reduces the always heavy burden of keeping alive cultures of organisms which are not in immediate demand for actual experiment. Some will also remain alive a long time when sealed airtight. Particular organisms may be kept a long time in particular media, *e. g.*, *Bacterium vascularum* in diluted peptonized cane-juice gelatin, *Bact. Stewarti* in milk, etc. Some organisms are quite resistant to their own decomposition products, *e. g.*, *Bacillus coli*, *Bact. pericarditidis*. In the cool box *B. coli* will often live a year in agar stab cultures.

MIXED CULTURES AND MIXED INFECTIONS.

The behavior of mixed cultures and mixed infections may be tested in various fluids, making poured plates from time to time; in tubes of agar, potato, and other solid media; in crossed streaks on agar or gelatin plates; and in the plants themselves.

When two bacteria, or a bacterium and a fungus, are sown together in a culture-medium, there may be (1) antagonism, with the crowding out of one species; (2) a more or less complete indifference, both organisms growing well; or (3) a distinctly favorable effect, *i. e.*, a marked increase in growth or in pathogenic effect due to the presence of the second organism. The antagonism may result in the prompt destruction of one of the organisms, or only in a retardation or inhibition which finally disappears after the first organism has made its growth and subsided. In some cases the favorable effect of one organism upon another is due to the fact that it prepares food for it out of an unfavorable substratum, *e. g.*, maltose from starch.

In the plant one organism often paves the way for others which complete the destruction, *e. g.*, *Bacterium campestre* and *Bact. solanacearum* are often followed by soft white rots. Some of the latter, however, are able to make their way unaided, a fact observed and known to the writer for a white rot of the cabbage as long ago as 1896.

The simplest way of studying the antagonistic action of bacteria is by means of crossed streaks on agar or gelatin plates. These may be made either simultaneously, or one after the other has begun to develop. The action of the antagonistic organism may also be obtained by letting its products diffuse through a collodion sac into bouillon inoculated with the other organism. In practice, the bottom of a test-tube is removed and a collodion sac is securely fastened in its place. This tube is filled with the usual quantity of bouillon and lowered into a larger receptacle (tube or flask), the collodion part being surrounded by bouillon. The inner and outer receptacles are now plugged with absorbent cotton, and the apparatus is sterilized in the steamer or autoclave. The two tubes are then inoculated simultaneously, or the outer one some hours or days after the inner one. (See an interesting paper on Antagonism, by Frost, in Jour. Infect. Diseases, Vol. I, 1904, pp. 599–640). Frost has also devised two new methods for studying this subject, viz, the divided-plate method and the agar-block method. The first is a modification of the ordinary streak method. It is managed as follows: A Petri dish is divided into two equal parts by means of a glass rod fastened to the bottom with collodion. A tube of melted agar is inoculated with the antagonistic organism and poured into one half of this plate. Into the other half sterile agar is poured. Streaks of the other organism are now made crosswise of the hardened surface. If there is marked antagonism there will be a decided difference in the behavior on the two sides of the plate, *i. e.*, on the sterile agar as compared with the inoculated. To insure a uniform streak the inoculated loop should be swept across one half of the plate, then reinoculated aud swept across the other half of the plate.

The method by agar-blocks consists in substituting agar-walls for collodion walls. A sterile 3-cm.-deep Petri dish is poured full of nutrient agar. When it has solidified it is cut into rectangular blocks, 1 by 1 by 3 centimeters, using a sterile knife and taking all possible precautions to avoid contamination by air-borne organisms. A platinum needle is now dipped into a culture of the supposed antagonistic organism and thrust into the block lengthwise but not entirely through it. The mouth of the needle-track is sterilized and sealed by touching it for a moment with a red-hot iron. The head of a small wire nail set into a suitable handle will answer the purpose. The block is picked up with sterile forceps and dropped into a tube of sterile bouillon, which then may be inoculated with the other organism. More than one block and tube should be inoculated, and it is best to test the sterility of the outer surface of the agar-block by delaying the inoculation of the bouillon for a day or two after the inoculated agar-block has been dropped into place.

Still another method has been described by Frankland and Ward. They use the walls of a Chamberland filter to keep the bacteria separate. Bouillon for the one organism is placed in a flask or large tube. That for the other organism is placed inside a Chamberland filter, which is then sunk into the other receptacle, whereupon it is sterilized and inoculated as in the collodion-sac method.

The favorable influence of a second organism may be studied in crossed streaks on sterile raw potato, carrot, turnip, etc.; on starch jelly; or on agar, gelatin, or silicate jelly with addition of varying amounts of the different plant acids, or plant juices, or other vegetable substances. Frost's divided Petri dish may be used for the jellies.

REACTION TO ANTISEPTICS AND GERMICIDES.

Antiseptic has been defined recently by Duclaux as follows: Any substance the intervention of which modifies in any form whatsoever the march of the phenomena (Bibliog., XX, Fermentation alcoolique, p. 461).

I still use the word with its old primary meaning (*anti*, against, and *sepsis*, decay). In this sense an antiseptic is any substance which prevents the multiplication of bacteria in putrescible substances. Large doses of antiseptics often exert a germicidal action, but such action does not necessarily follow. Often when the antiseptic substance is removed or diluted beyond a certain point growth takes place. The first seven substances mentioned below possess very active germicidal powers and are antiseptic in correspondingly small doses; the remainder are more or less valuable antiseptics, but are not valuable germicides.

(1.) Mercuric chloride.
(2.) Sulphate of copper.
(3.) Formaldehyd (formalin).
(4.) Phenol (carbolic acid).
(5.) Lysol.
(6.) Trikresol.
(7.) Methyl violet (Pyoktanin).
(8.) Thymol.
(9.) Benzoic acid.
(10.) Salicylic acid.
(11.) Chloroform.
(12.) Sulphuric ether.

This list may be extended indefinitely. The student should consult valuable digests in Sternberg's Text Book of Bacteriology and in Miquel & Cambier's Traité de Bactériologie. Some caution must be used in drawing conclusions from experiments. Mercuric chloride does not always destroy when the culture medium contains albuminoid substances. Sulphate of copper is more active in water than in bouillon.* Some organisms will grow in a solution saturated with thymol (*e.g.*, in bouillon). Others will grow in the presence of chloroform (5 cc. of chloroform in test-tubes with 10 cc. of milk or beef-bouillon). Ten organisms have been found by the writer which, under the conditions named, grew in the presence of chloroform and two which grew vigorously in the presence of thymol. Russell reports one capable of growing in the presence of sulphuric ether. It is, therefore, not always safe to depend on these substances as antiseptics. Newcombe has made the same observation (Cellulose Enzymes, Annals of Botany, Vol. XIII, 1899, p. 60). In the opinion of the writer the statements of physiologists respecting the existence of enzymes in the tissues and fluids of the higher plants and animals must be taken with much allowance when chloroform, thymol, and similar antiseptics have been

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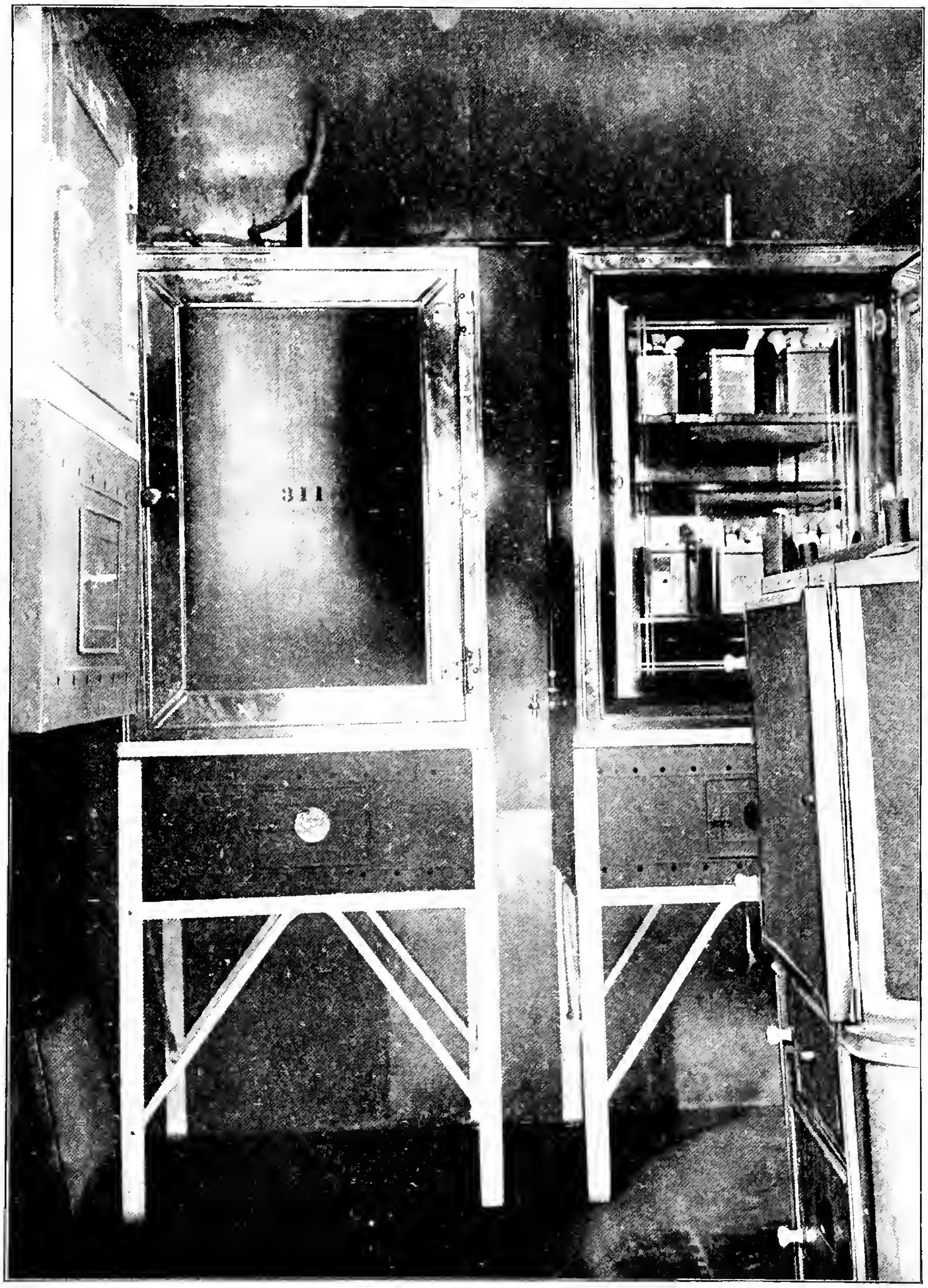
*Moore, George T., and Kellerman, Karl F. A Method of Destroying or Preventing the Growth of Algae and Certain Pathogenic Bacteria in Water Supplies. U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin 64, 1904, pp. 44; see also Bull. 76, Bureau of Plant Industry. Certain pathogenic bacteria, such as *Vibrio cholerae* and *Bacillus typhosus,* are destroyed within a few hours in water containing traces of copper salts or dissolved particles of metallic copper.



A thermostat-room.

In the center of the building and lighted by electricity. Ventilated in the same way as the photographic dark-rooms, i. e., by an exhaust-fan run by an electric motor. Three of the thermostats were made by Bausch & Lomb, the fourth (felted) is a Rohrbeck.

depended upon to keep the solutions free from bacteria. This has been the case very frequently, and in several places in Greene's interesting book on Fermentations, published in 1899, it is said or inferred that the addition of chloroform will prevent the growth of bacteria. This might or might not be true; much would depend on the kind of organisms present. The medium to which chloroform or thymol has been added must be shut in and shaken continuously if the full antiseptic value of these substances is to be obtained.

THERMAL RELATIONS.

The student should determine—

(1) Maximum temperature for growth (thermostat).

(2) Minimum temperature for growth (ice-box).

(3) Optimum temperature for growth (room or thermostat).

(4) Thermal death-point (ten minutes exposure in the water-bath, in thin-walled test-tubes of resistant glass having a diameter of 16 to 17 mm., ordinarily in 10 cc. of moderately alkaline peptonized beef-bouillon, viz, +15 of Fuller's scale).

(5) The effect of freezing (exposure to liquid air or to pounded ice mixed with coarse salt).

Thermal relations are among the most interesting and should be studied with great care in case of every organism. They offer valuable means of differentiation and also very useful suggestions as to geographical distribution and habitat. Good thermostats are made by various people. Several items of construction are important. The water or oil jacket should be of considerable volume (thickness) so as not to change temperature quickly; the cover should be thick and of the best non-conducting substances. The opening for the thermo-regulator should be at least 1½ inches in diameter (so as to take a Roux metal-bar thermo-regulator); the warm chamber should be of good size; the space beneath should be high enough between floors to accommodate any pattern of safety burner; and last, but not least, the workmanship should be of the very best quality, so that the apparatus will not leak. Nearly every worker has probably had experience with leaky thermostats at some time in his life and knows what a vexation of spirit they cause, particularly if filled with oil. A very excellent kind of thermostat is the old, large-pattern, felt-covered instrument devised by Dr. Hermann Rohrbeck and figured in the lower right-hand corner of plate 8. This plate shows a thermostat room with four thermostats in use. All are provided with Roux metal-bar thermo-regulators and Koch safety burners. One is for quick shifts as needed; and others are generally kept at 30°, 37½°, and 40° or 43° C. These temperatures, in conjunction with the cool boxes, thermal baths, and various room temperatures, enable one to quickly determine the thermal relations of an organism. The height of the room is 10 feet, its depth 7 feet, and its breadth 5 feet 3 inches. A larger room would be more convenient. Such a room should be located and constructed so as to be as little subject as possible to external changes of temperature. It should be lined with asbestos and sheet iron, and *efficient* safety burners should be used to the exclusion of all others (see Lautenschläger's catalogue). The improved Koch safety burner is probably the best. All burners require frequent inspection.

The writer has no very satisfactory way of making exposures for determining the minimum temperature for growth. His method is to make such exposures in the bottom of a large, well-filled ice-box, which is opened as little as possible during the progress of the tests, and then only for the briefest periods. The degree of cold is governed by the amount of ice. A good thermometer is exposed in the midst of a bundle of inoculated tubes, and if the temperature shows any tendency to rise more ice is added. Under the most favorable circumstances the temperature of the

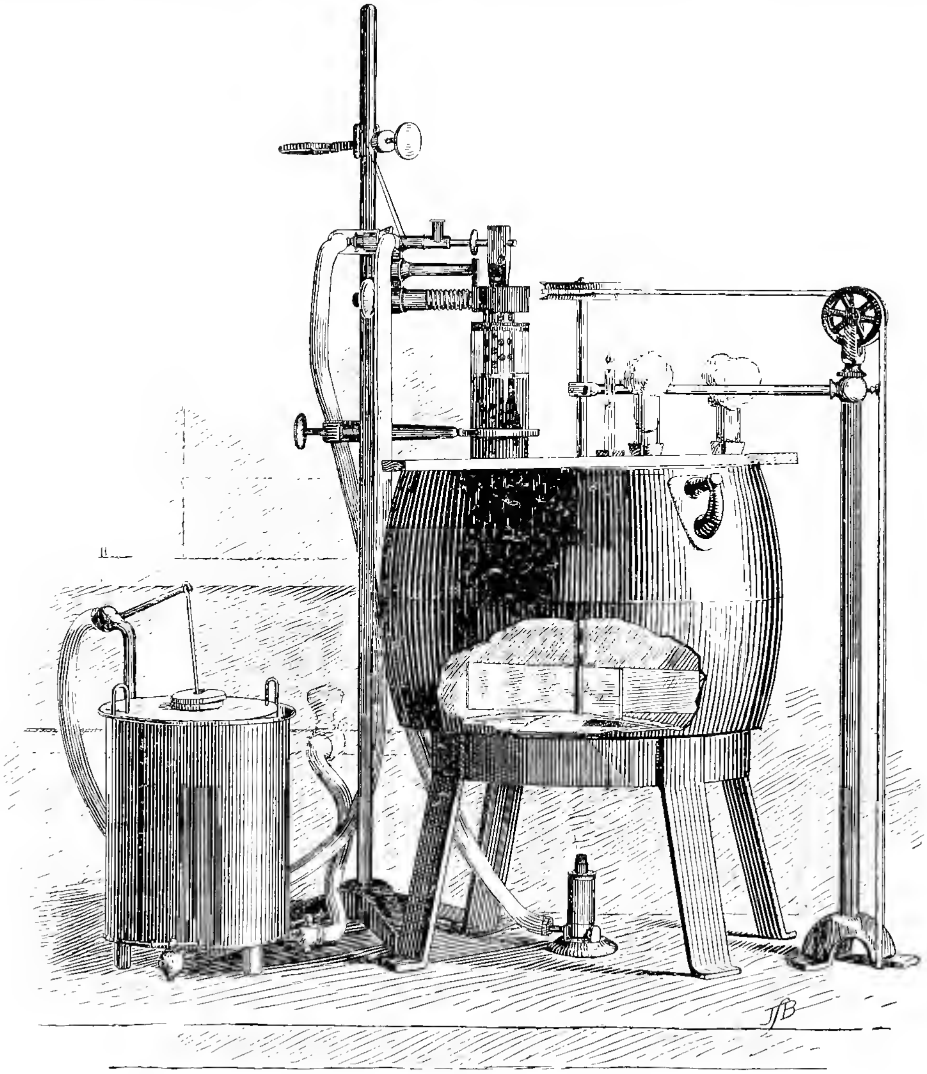


Fig. 63.*

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*Fig. 63.—Modification of the Ostwald water-bath used by the writer for thermal death-point experiments. This consists of a porcelain-lined pot 11 inches in diameter at the top. This is filled with water kept in motion by a water-wheel turned by electricity. The heat is applied by means of a Friedburg burner and is controlled by Roux's thermo-regulator. Murrill's gas-pressure regulator is shown at the left.

air in the bottom of the chest may be kept fairly constant for some days or weeks, but with marked external fluctuations of temperature trustworthy results can be obtained only by constantly watching the box. What one needs for this work is a good-sized room kept at 0° C., or a little below, in which thermostats may be installed at temperatures a little above freezing, *e. g.*, +2°, +5°, +7°, etc. It would then be very easy to determine the minimum temperature at which any organism will grow—as easy as it is now to determine the maximum. Different levels in the same room may afford constant and useful differences in temperature.

The thermal death-point, which is a purely arbitrary standard, depending on the age and kind of culture, its volume, and the length of exposure, as well as the temperature, is when properly determined not least valuable. The writer, following that one of Dr. Sternberg's methods which is easiest to carry out, uses 10 cc. portions of moderately alkaline (+10 or +15) peptonized beef-broth† in test-tubes of uniform diameter (16 to 17 mm.), inoculates from recent bouillon-cultures with care not to touch the sides of the tube above the fluid, thrusts the tubes deep into the hot water, and exposes for ten minutes. All who make this test are urged to use standard alkaline beef-bouillon (for all organisms growing well in this medium) and to limit the exposure to exactly ten minutes, so that easy comparisons may be made. The five minutes exposure which has been recommended by some authors is rather too short, since it only a little more than suffices to warm the fluid up to the required temperature. Inoculation while the tubes are in the bath and after the fluid has been brought to the required temperature is inconvenient and has no special advantage.

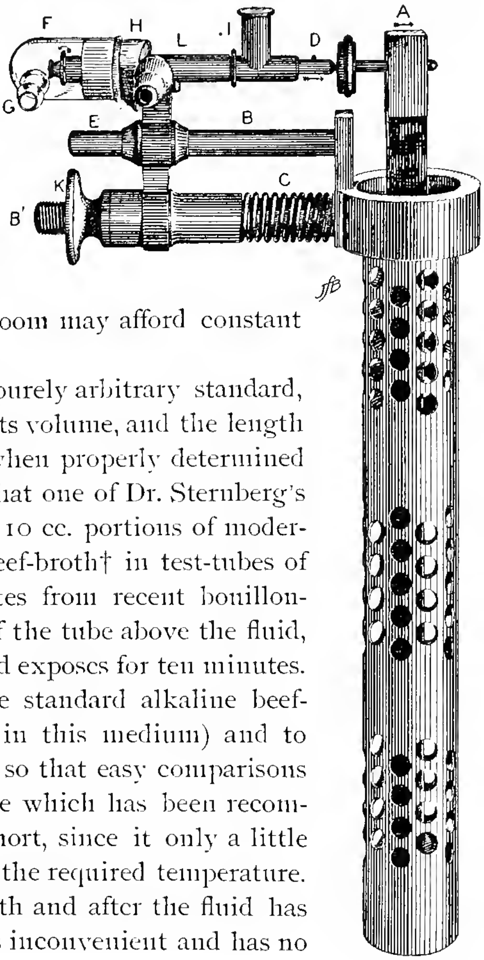

Fig. 64.*

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*Fig. 64.—Roux's thermo-regulator, made by Maison Wiesnegg (P. Lequeux), Paris. The parts requiring description are as follows: A, bar composed of two metals (which expand and contract unequally) attached at bottom and free at the top, which moves with increased heat in the direction of the arrow; B, arm on which the upper part of the apparatus moves freely when K is turned; C, stiff spring; D, long rod which controls the gas-inflow, and the spring movement of which is in the direction of the arrow except when controlled by the counter movement of A, due to lessened heat; E, gas-inflow; F, gas-chamber, of glass; G, gas-outflow, to the burner; H, rubber stopper; I, cylinder screwing into L, and provided with capped upright tube filled with vaseline to prevent gas from escaping in the direction of D. The button shown in the gas-chamber at the left is part of D, and the gas enters the chamber between it and the left end of L, the size of the opening, and consequently the amount of gas, varying with the slightest movement of A. Different temperatures are obtained by turning the button K. The constant gas-flow is provided for by a small opening on the lower side of L at its extreme left, in the gas-chamber. About two-fifths actual size.

†The thermal death-point in acid media is considerably higher—at least that of several organisms which have been tested in the author's laboratory.

An excellent water-bath is that known as the Ostwald-Pfeffer. The experimenter may, however, construct one for himself out of a medium-sized, thick-walled, porcelain-lined iron kettle (fig. 63). This should rest on a ring of heavy strap-iron supported by four stout iron legs. The burner required may be Dr. Friedburg's safety burner (a very inexpensive and good pattern). The thermo-regulator may be a common Reichert if the mercury seal is cleaned from oxide frequently. In such regulators a sharper contact and a longer freedom from obstruction is said to be obtained (Dr. Harris) by putting a drop of olive oil on top of the mercury. A much better instrument is the metal-bar mechanism known as the Roux regulator (fig. 64). This may be procured from the Maison Wiesnegg, in Paris. It should be kept from direct contact with the water and consequent rusting by burying it in a close-fitting glass tube filled with olive oil or glycerin. This tube is then sunk deep into the water and clamped to the wall of the kettle, which should have perpendicular sides. The water is kept in motion by means of a horizontal paddle-wheel at the bottom of the kettle. This consists of four light, oblique zinc or copper vanes (nickeled copper is preferable) soldered to a long central rod which fits into a socket, below, and near its upper end passes through a hole or loop in a horizontal metal arm (a foot or less above the kettle), the other end of which is clamped to the upright rod of a solid iron tripod, or fastened to a rod bolted to the table. If compressed air can be had, a stiff cardboard windmill fastened to the upper end of the vertical rod completes the mechanism. The central part of the wind-wheel may be of cork. The vertical rod may be a piece of glass tubing, in which case it is cemented into a socket of the short metal post to which the vanes of the water-wheel are soldered. If a wind-wheel is attached, it is more convenient to have the vertical rod in two parts, fastened by a coupling. The rod, with its water-wheel attachment, may also be turned by some electrical device. The latter is the most convenient method. In fig. 63 the electric motor is not shown. This stands in a small box screwed to the under side of the table at the right. The switch is fastened to the wall above and back of the top of the thermo-regulator. The pulley band is of smooth rounded leather one-eighth inch in diameter. The electric current is passed through an Edison lamp screwed under the table to reduce the velocity of the motion. With the lamp in place and the current cut down to the minimum the number of revolutions per minute is 55, and the temperature of the water is the same in all parts of the bath. The simplest contrivance of all is to make the water-wheel and upright shaft of wood, to be turned by hand.

In localities where the gas-pressure is exceedingly variable, Paul Murrill's gas-pressure regulator (at the left in fig. 63) will be found useful. This is made by Eberbach & Co., Ann Arbor, Mich. (see Journal of Applied Microscopy, Vol. I, p. 92, or Centralb. f. Bakt., 1 Abt., Band XXIII, 1898, p. 1056.) The gas-pressure may be somewhat improved by simply passing the gas through a big bottle (see top of thermostat 311 in plate 8). The Anschütz normal thermometers, with long stem and scale divided into fifths, are very convenient for determining temperatures (fig. 65). They come in sets of seven, but may also be had separately. The most frequently useful are No. 1 (scale −15° to +55°) and No. 2 (scale +45° to +105).

They cost 9 marks each when ordered direct from Berlin, and can be had without delay. Good American thermometers are made by Henry Green, New York.

With this open bath it is easy to keep the range of temperature down to 0.1 to 0.2 of a degree, and the writer has frequently exposed tubes for ten minutes without appreciable change in temperature. Temperatures may be read easily to 0.1 degree by means of a Zeiss aplanat lens magnifying six times (fig. 25), and should be recorded for each half minute during the exposure. Under no circumstances should exposures be made in water which is not agitated. Of course, for accurate reading the eye and the center of the lens must be level with the top of the column of mercury. The lens may be supported at the proper level on a grooved piece of cork. If possible the thermometer used should be compared with some standard instrument. If not, it should at least be compared with several other good thermometers in the same laboratory. The test-tubes are supported by perforated corks thrust into holes bored through a rectangular piece of hard, *heavy* wood.

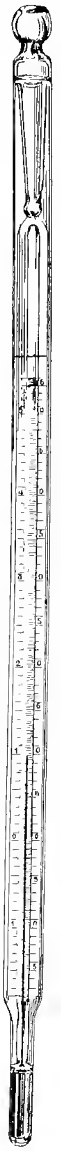
Fig. 65.*

The writer formerly made use only of the first four tests. It seemed hardly worth while to recommend that all bacteria be tested for the killing effect of cold, so long as we had nothing but the inconvenient and more or less inexact methods of salt and pounded ice or of ether and frozen CO_2; but now that liquid air may be obtained at a small price in many of the larger cities, can be shipped long distances, and can be used with so little inconvenience, there is no good reason why the effect of freezing should not be determined in all cases, since in some instances it is likely to prove a valuable means of differentiation. The bacteria may be exposed in 5 cc. portions of distilled water or bouillon in block-tin test-tubes, or preferably in tubes of resistant glass, for standard periods, *e. g.*, one-half hour, 1 hour, 6 hours, 12 hours, 24 hours, 48 hours, etc. They may also be exposed to alternate freezing and thawing every fifteen minutes or thirty minutes until all are dead. To avoid endospores, the depressing effect of by-products, etc., young cultures should be used, and, of course, all should be of the same age and grown in the same medium, *i. e.*, bouillon cultures 24 hours or 48 hours old. The tests should be quantitative rather than qualitative. They may be made as follows: Into 5 cc. of sterile water or standard bouillon a carefully-measured quantity, *i. e.*, one loop, 5 drops, ½ cc., etc., of the culture is placed, stirred very thoroughly, and allowed some time for diffusion. To avoid zooglœæ, which form early in some species, and to reach more uniform measurements, it is recommended to take the loop from a bouillon culture rather than from agar or other solid media. After sufficient time has elapsed for uniform diffusion, six Petri-dish poured plates are made from each of the inoculated tubes. The plates should be of the same diameter (area of 60 sq. cm.). The amount of agar used for each plate should be 10 cc., and the amount of infectious material used should be the thinnest obtainable film of fluid across a carefully-measured 1 mm. loop, so as to avoid crowding the plates. The same loop should be used in all cases, and it should be dipped into and out of the fluid always in the same way. After pouring, set the plates on a perfectly level spot (fig. 66), until the agar has hardened. If the work has been well done, there should develop an approximately uniform number of colonies in each plate. The tubes of inoculated water or bouillon are then immediately lowered into the liquid air and exposed to it for the predetermined time, after which six additional Petri-dish poured plates, of the same size and inoculated in the same way, are made from each tube for comparison with those prepared before the exposure. The tubes may be thawed out by exposure to the air for three minutes and then to tap-water for five to seven minutes. The exposures are best made in Dewar glasses (fig. 67). When the exposures are long, a loose tuft of absorbent cotton should be placed in the mouth of the glass, or it should be covered with a hair-cloth cap, to prevent excessive evaporation. Under these conditions the air remains liquid for a number of days. At first the temperature is about minus 190° C., rising gradually to minus 180° C., since the nitrogen evaporates somewhat faster than the oxygen. The glasses are fragile and should be handled carefully, especially when filled with the air. As long as they contain liquid air it is safer to keep them in their containing-case, packed about with cotton or felt. One should be careful to avoid cracking the inner wall of the glass, as might happen by dropping some hard substance into the receptacle, otherwise an explosion will occur, the space between the two walls of the Dewar glass being a very perfect vacuum.

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*FIG. 65.—Anschütz normal thermometer with degrees divided into fifths (Centigrade scale). For use in thermal death-point tests. About three-fourths actual size.

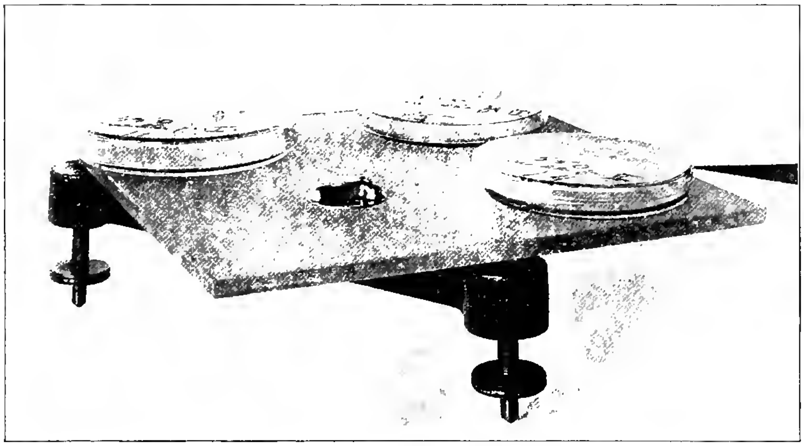

Fig. 66.*

When the exposures are made in block-tin tubes, the culture should be frozen at once on pouring into the tube and the second set of plates should be made as soon as the fluid has thawed, *i. e.*, within about ten minutes, for which purpose the culture should be poured out into a glass tube, otherwise complications due to the germicidal action of the metal might arise. In no case should the cultures be incubated in the tin tubes. When exposures are made in test-tubes of resistant Jena glass, the cultures must be lowered into the liquid air gradually, the fluid being frozen from the bottom upward to avoid cracking the tubes. It requires about four minutes to properly freeze a culture in a glass test-tube. Large volumes of culture media should not be lowered into the liquid air, as it is wasteful, the air boiling away rapidly. The writer began his experiments with block-tin tubes, as shown in fig. 67, but now uses tubes of Jena glass. The latter crack occasionally in spite of care.

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*Fig. 66.—Leveling (nivelling) apparatus for use in making poured plates. About one-third actual size.

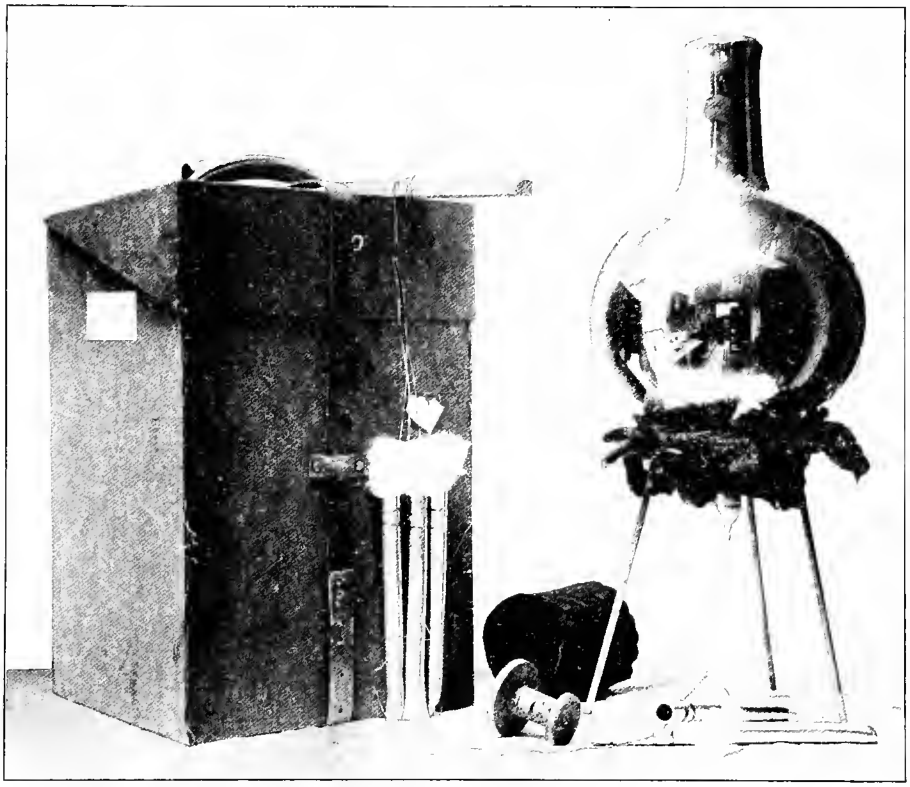

Fig. 67.*

For very rapid freezing the amount of fluid in the tube may be reduced to 1 cc. Liquid air in Dewar glasses, and compressed oxygen, hydrogen, and carbon dioxide (?) in steel cylinders may be had from the Eagle Oxygen Company, Incorporated, 121 West Eighty-ninth Street, New York City. The tanks of compressed gases may be bought or rented. The following sizes may be had: Fifty gallons (280 pounds pressure per square inch); 100 gallons (240 pounds pressure); 150 gallons (225 pounds pressure); and 200 gallons (280 pounds pressure). Cylinders may also be had with the gas under much greater pressure. The cost of the oxygen is 2½ cents per gallon. The wrought-steel cylinders cost about $10 each. A good quality of resistant-glass test-tubes may be had from Greiner & Friedrichs, Stützerbach, Germany. One sort has a faint-blue longitudinal stripe blown into the glass, another kind has the letter "R" etched on the upper part of each tube. Tubes without any distinguishing mark should not be purchased, as they are likely to become mixed with ordinary non-resistant tubes. The cost of these tubes, duty free, is about $16 per thousand. Good Petri dishes may be obtained from the same firm, and also from E. H. Sargent & Co., Chicago.

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*Fig. 67.—Dewar glass for liquid air, and block-tin test-tubes used in first low temperature experiments with bacteria. About one-sixth actual size.

The temperature demands of bacteria are extremely variable. Whole groups of them are able to live under conditions which would be impossible for the higher plants and animals. Many of the northern forms, especially those which grow in water, are adapted to low temperatures. The organisms of dung-heaps and thermal springs, and the tropical forms, often grow at high temperatures.

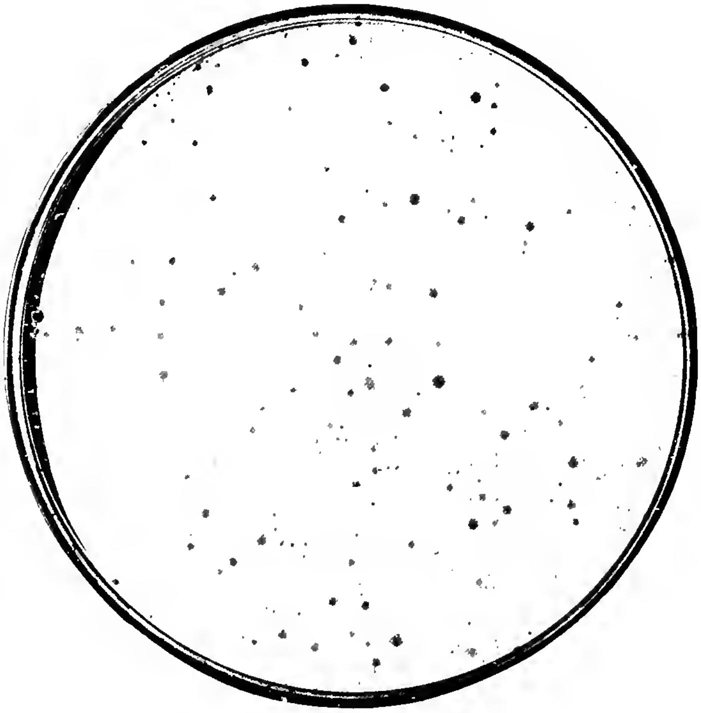

Fig. 68.*

For a very few species it has been known that prolonged freezing or repeated freezing and thawing destroys the weaker individuals and finally all. (See Bibliog., XXXIII, especially papers by Sedgwick & Winslow, and by Park; consult also an earlier paper by Prudden, Bibliog., XLVI.) For the bacteria as a whole, however, it has been assumed that ordinary freezing or even very intense cold simply inhibits

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[*] Fig. 68.—Petri-dish poured plate of *Bacillus tracheiphilus*. The 10 cc. of nutrient agar was inoculated with a carefully measured loop of a fluid culture. The fluid culture was then exposed in block-tin test-tubes to the temperature of liquid air, after which another plate (fig. 69) was made.

growth for the time being. Such statements have been based on certain qualitative tests and do not tell the whole truth. In the writer's experiments with liquid air great differences have been detected, the reduction by exposure for one-half hour varying from 15 per cent, or less, to 90 per cent, or more, according to the species tested. Fully 50 per cent of many sorts, grown in bouillon, are destroyed by a single short exposure (see figs. 68 and 69). Query: Is intense cold any more harmful to bacteria than simple freezing? Are young or old cultures most susceptible? Are they killed by the rupture of the cell-wall due to the formation of ice-crystals, or simply by the abstraction of water? Why do some resist several freezings? Can endospores be killed in this way? Consult '01, d'Arsonval (Bibliog., XXXIII) and Smith & Swingle, the Effect of Freezing on Bacteria, Proc. Sixth Ann. Meeting Soc. Am. Bacteriologists, December 27, 1904; Science, N. S., Vol. XXI, 1905, pp. 481-483. For opposing views see '02, Macfadyen, Bibliog., XXXIII.

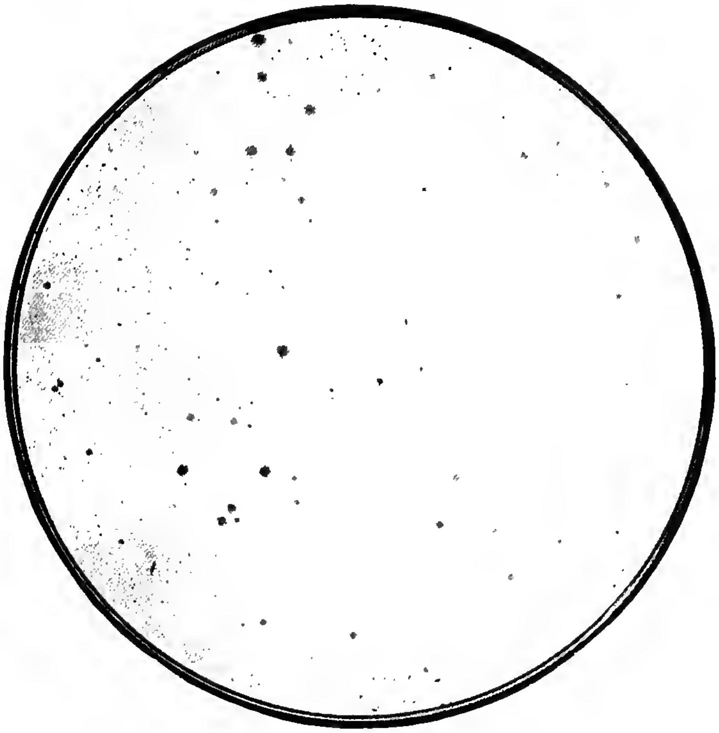

Fig. 69.*

Live steam acts upon the growing bacteria very quickly. All bacteria not in spore form, or in some other way protected from the direct action of the heat by what surrounds them, are promptly destroyed by steam heat at 100° C., an exposure of a minute or two being ample, except, possibly, in case of some of the thermo-

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*Fig. 69.—Same as fig. 68, but made after exposure for twenty hours to liquid air. Number of colonies reduced two-thirds. Exposed in test-tubes of Jena-glass for one-half hour, the reduction was nearly as great, *i. e.,* over 50 per cent. In this latter case the agar plates were incubated 7 days at 30° C, before the count was made.

philic species. Usually even the most resistant spores, if freely exposed, are destroyed by one to two hours exposure to 150° C., of dry heat, or by thirty minutes exposure on each of three consecutive days to streaming steam at 100° C. Some very resistant spores have survived a single steaming or boiling of five or six hours duration (eight hours in one of Tyndall's experiments), and it is not unlikely that some slowly germinating sorts may be able to resist discontinuous steamings for three days. It is possible also that there may be some sorts able to germinate and again assume a resistant spore form in less than twenty-four hours although this is not probable. Some spores are destroyed by a short boiling at 100° C., and all spores are quickly destroyed by steam under pressure, *i. e.*, in an autoclave. A temperature of 110° C. for ten or fifteen minutes is sufficient. Exposure of media to higher temperatures and for longer periods should be carefully avoided. It must be remembered, however, in using autoclaves, that all of the air must be replaced by steam before the apparatus is closed, otherwise the temperature to which the medium is exposed will not correspond to that indicated by the pressure gage. The most convenient autoclaves known to the writer are the large sizes of the

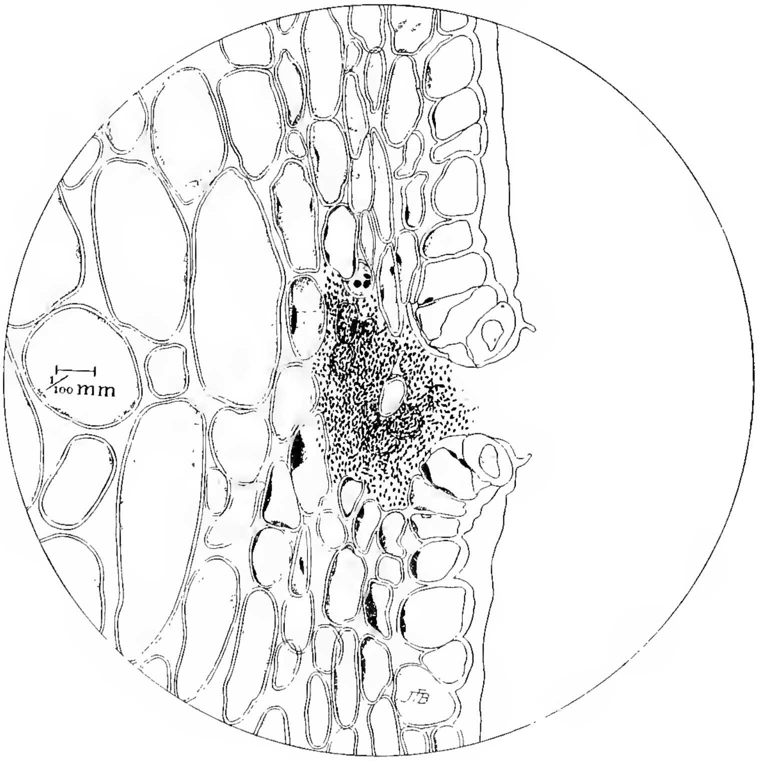

Fig. 70.*

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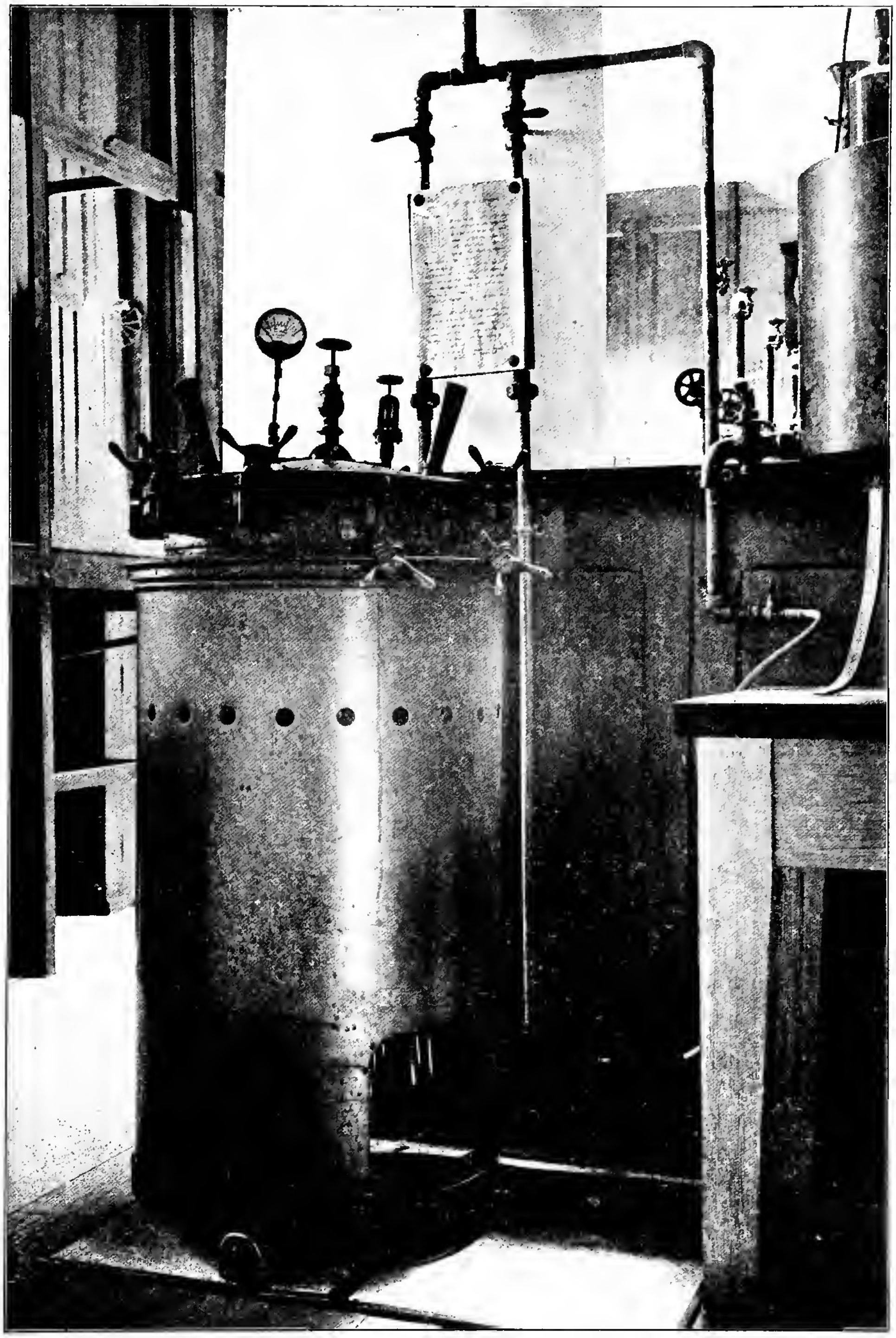
*Fig. 70.—Earliest stage of fruit spot on green plums, due to *Bacterium pruni* (Erw. Sm.). The bacteria have entered through the stoma. They disappear farther in, and also a few micra to either side of this stoma, as shown by an examination of the serial sections. Material fixed in strong alcohol, infiltrated with paraffin, and cut on the microtome in series. Section stained with carbol-fuchsin and drawn directly from the microscope with the aid of a camera lucida.

PLATE 9.



Chamberland autoclave.

Heat is applied to the bottom by means of a double ring of Bunsen burners. No wrench is required for fastening on the top. About one-eighth natural size.

pattern designed by Chamberland and made by the Maison Wiesnegg (P. Lequeux), Paris, France, the steam being generated by gas (plate 9). The steam gage is at the left; in the middle is the valve through which the hot air is allowed to escape when the instrument is warmed up; at the right is the steam safety-valve. The temperature is manipulated by regulating this valve. By leaving the vent open the apparatus may be used as an ordinary steam sterilizer. It may also be used as a distilled-water apparatus by attaching a condenser to the exit pipe of the middle vent, but such water must not be used for culture media. A very good autoclave is also made by the Kny-Scheerer Co., New York. Harding recommends for autoclaves the use of steam from the engine-room boiler. This is convenient, provided one can always have steam ready during the summer months. An autoclave, like a steam boiler, which it is, must be watched carefully if it is not some time to explode from excess of heat or lack of water. Each time before use one should see that the apparatus contains sufficient water.

Soils are rather difficult to sterilize. They may be spread in thin layers and dry-heated for several hours at 150° C., or may be heated in the autoclave for an hour under a pressure of two atmospheres, taking care to drive all the air out of the soil before closing the apparatus. It is not likely, however, that soils can be treated in this way without undergoing certain physical and chemical changes. Small pots of soil may be heated in the steamer at 100° C. for two hours on each of five successive days.

The reason for preparing all media in the autoclave, or by heating in the steamer at 100° C. on three successive days (the ordinary way), is because we are never certain in what particular case resistant spores may be present. One short steaming is often sufficient to sterilize media prepared in a cleanly way, as every bacteriologist knows who has had much experience, but now and then, in spite of all care, resistant spores will find their way into culture media, and for this reason it is best in all cases (especially in teaching students) to adhere to a routine of three steamings. Large masses of fluid (beakers, flasks) require longer steamings than test-tube cultures. The writer gives double time, or triple time. Discontinuous boiling as a means of sterilization was introduced in 1877 by Tyndall, who well says respecting the sterilization of liquids: "Five minutes of discontinuous heating can accomplish more than five hours continuous heating."*

Most plant-pathogenic bacteria of temperate and cold regions have a lower optimum and maximum temperature for growth and a lower thermal death-point than species pathogenic to warm-blooded animals. The maximum temperature for growth is usually at or below 36° C. We should not, however, expect this to be true of bacterial plant parasites in tropical and sub-tropical regions, about which, however, little is known beyond the mere fact that such parasites occur. Savastano states that the optimum temperature for the olive-knot organism, which is said to be more prevalent at the southern than at the northern limit of olive-growing,

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*This method appears to have been known to housewives for a much longer time. In Dr. Samuel Johnson's Dictionary (first Am. from eleventh London ed.) I find the following definition: "Biscuit, A kind of hard, dry bread made to be carried to sea. It is baked for long voyages four times."

i. e., commonest in southern Italy, Sicily, and Algeria, lies between 32° and 38° C. In my own experiments with this organism, obtained from olive trees in California, I have found its maximum temperature to be above 35° and below 37.5° C. The optimum temperature of *Bacterium solanacearum*, which is very destructive to potatoes and tomatoes in the southern United States, is probably about 35° C.—at least it grew readily and remained alive for a long time in bouillon kept at 37° C. Its maximum temperature is 39 +° C. *Bacillus carotovorus*, one of the best known of the soft-rot organisms, grows well in the thermostat at 33° to 34° C. Its maximum temperature is at 39° C. or slightly below (Jones). *Bacillus aroideæ*, whose temperature relations were recently studied carefully by Townsend, has a maximum temperature of 41° C. A temperature of 40° C. retards growth, but does not prevent it. This organism was isolated from calla-lily corms, but is capable of causing a soft rot in potatoes, carrots, turnips, and many other plants (fig. 102). The maximum temperature of *Bacillus oleraceae*, recently described by Harrison, is said to be about 42° C. This causes a soft rot of cauliflower.

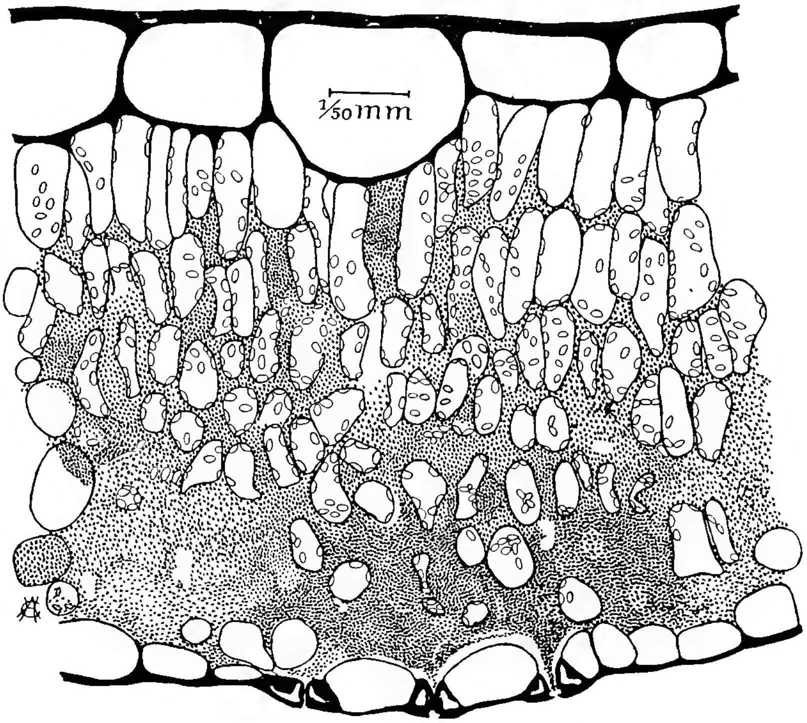

Fig. 71.*

The range of temperature suited for the growth of particular bacteria varies greatly. Some species are able to grow through a range of 50° C. Many tolerate a range of only about 30° C. Certain animal-pathogenic forms have through long subjection to a peculiar environment become restricted to a still narrower range.

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*Fig. 71.—*Bacterium pruni.* Early stage of a leaf-spot in the plum. The small spot was water-soaked in appearance, but it had not yet collapsed. The bacteria, which are most abundant in the mesophyll, undoubtedly entered the leaf through the stomata, three of which are shown in the section. Material treated as in fig. 70. Section drawn with the aid of an Abbe camera. It represents as nearly as possible one plane.

Some bacteria grow well only in the cool box, others only in the thermostat at blood-heat or at higher temperatures,—temperatures elevated enough to quickly destroy the unprotected protoplasm of the higher plants and animals. Few of the bacteria commonly studied will grow at temperatures much above 40° C., but this by no means expresses the whole truth.

The *lowest temperature* at which growth will take place ranges in different species all the way from 0° C., and probably a few degrees below (certain salt-water bacteria) to + 40°, + 50°, + 56°, and even + 60° C. (certain thermophilic species found in dung-heaps, hay-mows, silos, hot springs, etc.). The *highest temperature* at which growth will take place ranges from as low as 30° C. (and probably lower*) to as high as 75°, or 80° C., or even 89° C., according to Setchell. Higher temperatures have been recorded, but I have here used only those determined with care in the exact places frequented by the bacteria. This will be better appreciated if it is remembered that a temperature of 60° C. (140° F.) can be endured by the fingers only a few seconds, while 70° C. (the optimum for some of these species) is intolerable to the hand even for the shortest period. It seems incredible, on first thought, it is so opposed to our customary observations, that any organism whatsoever should be able to live at a temperature only 11 degrees below the boiling point of water Nevertheless, protoplasm is an extremely adaptable substance, and it is conceivable that some organisms might grow at a temperature considerably higher.

The thermal death-point (10 minutes exposure) ranges from 43° C. for *Bacillus tracheiphilus*, the lowest yet recorded,† to temperatures only a few degrees under the boiling point (100° C.). For many species the thermal death-point lies between 50° and 60° C. Russell & Hastings have recently discovered in milk a Micrococcus whose thermal death-point is 76° C.

As the upper and lower thermal boundaries of growth are approached some functions are extinguished in advance of others; *e. g.*, pigment production, pathogenicity, and sporulation disappear considerably in advance of loss of power to reproduce by fission.

OTHER HOST PLANTS.

Plants of related species, genera, and families should be tested. If the disease appears to be new to literature, it is also especially important to inoculate those plants which have been reported to be subject to bacterial disease and the nature of which disease is still in doubt. Many facts of scientific and economic interest will be brought to light in this way, and now and then the experimenter may be able to clear away some of the fog which, owing to the uncertain and contradictory statements of a majority of our plant pathologists, still hangs over the origin and nature of most of these diseases.

Some plant pathogenes appear to be quite narrowly restricted. They attack only one host plant, or at most a few hosts belonging to related species or genera. Others, particularly some of the soft-rot bacteria, attack many kinds of plants belonging to widely different families. The history of pear-blight, however, shows us that

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*Since this was written Molisch states (l. c., p. 93) that gelatin cultures of his *Bacterium phosphoreum* were dead at the end of 48 hours when exposed to a temperature of 30° C. The maximum temperature of this organism is said to be about 28° C.

†Very recently Marsh has found a fish parasite which is said to have a thermal death-point of 42° C. (See VI, Bibliography of General Literature.)

the restriction of an organism to a single host-plant may be only an inference based on insufficient observation rather than an actual fact. After a time the apple and quince were added to the pear as host-plants, and now we may add also the plum and the loquat.

PATHOGENIC OR NON-PATHOGENIC TO ANIMALS?

If the organism will not grow in the thermostat at 37° C., or grows only feebly, as is the case with many plant parasites, it may be assumed to be non-pathogenic to animals with warm blood. Only those organisms which grow readily in the thermostat at 37° C., and which closely resemble animal-pathogenic forms or which are suspected of causing some particular disease of animals, need be tested by animal experimentation for economic purposes. In general, it is best to leave this part of the work to the animal pathologist, for the same reason that the more abstruse chemical problems are turned over to the chemist.

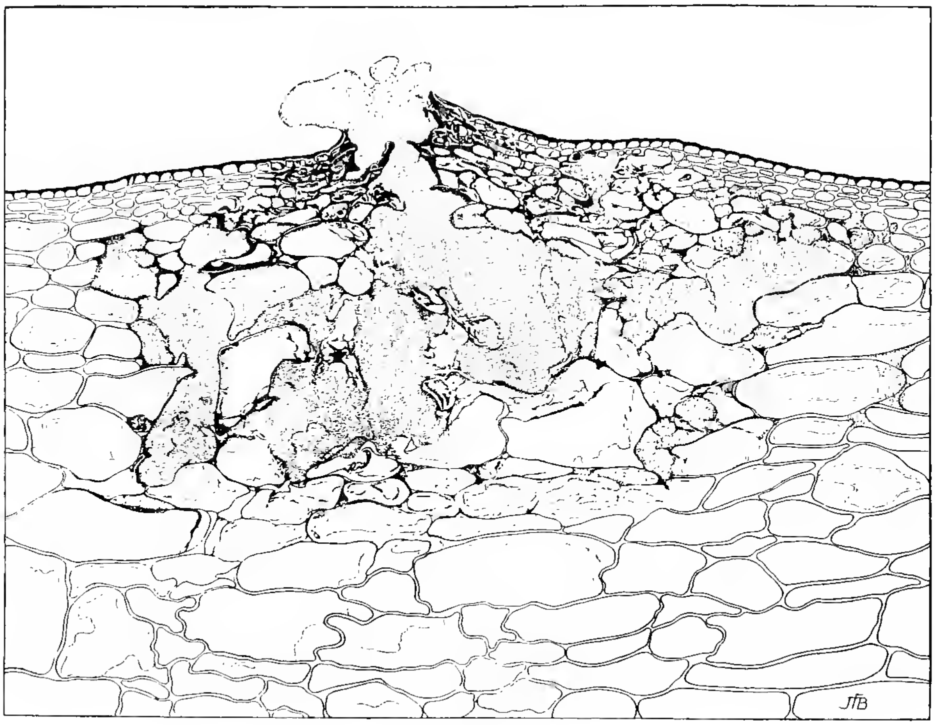

Fig. 72.*

All of the plant-parasitic bacteria, so far as tested, have turned out to be non-pathogenic to warm-blooded animals, but it is not unlikely that some exceptions may be discovered.

Another question, of special interest to animal pathologists, arises here, namely, whether forms known to be pathogenic to animals and especially to man are ever

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*Fig. 72.—*Bacterium pruni.* Vertical section through a green plum fruit (var. Hale) showing bacterial cavities and the escape of the organisms through the ruptured stoma. In this case beyond doubt the central stoma is the one through which the infection originally took place. Drawn from a photomicrograph. The material was fixed in alcohol, infiltrated with paraffin, cut on the microtome, and differentially stained.

harbored by plants. Of those known to cause animal diseases none have ever been found naturally present in plants, but some of them, such as the typhoid bacillus, the anthrax organism, etc., have been shown to live for a number of days or weeks when injected into various living plants, and in some instances have been found to multiply a little in the vicinity of the wounds. In general, their life is short in such situations, they do not penetrate far into the tissues, and they are manifestly on the defensive. If they can do no better when injected into vegetable tissues in enormous quantities, it seems rather unlikely that under ordinary natural conditions they would find their way into plants so as to make them dangerous for food. In this connection the reader is referred to Volume II, where this subject is discussed more fully. More danger is likely to result from pathogenic organisms carried on the surface of plants, especially on salads and fruits which are not cooked. In times of the general prevalence of typhoid fever, cholera, or the bubonic plague, the writer for one would certainly prefer to forego salads and to eat only freshly cooked vegetables. The danger from such foods in time of epidemics is very great, especially in localities where ditch-water is frequently sprinkled on the vegetables to freshen them, *e. g.*, in parts of southern Italy.

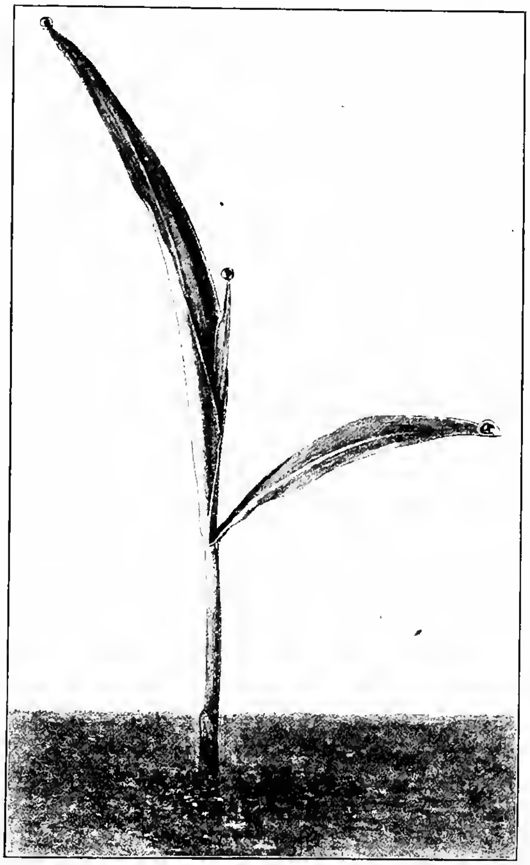

Fig. 73.*

Most saprophytes when injected into living plants behave in the same way as the animal parasites, *i. e.*, they either die at once or maintain a precarious existence for some weeks in the vicinity of the wound and then succumb. The writer has made many experiments, with negative results. The most extensive published series of experiments are those of Zinsser (Jahrb. f. wiss. Bot., 1897). To get a particular disease, the parasite must be used and not some other organism. This the writer has observed over and over again. This statement holds good with plants the same as with animals. In case, however, of the less typical plant diseases (soft rots) various members of a group of closely related organisms may produce essentially similar phenomena. This is paralleled, however, in certain of the less typical animal diseases.

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*Fig. 73.—Seedling sweet-corn plant extruding water from its leaf-tips. Most of the infections by *Bacterium Stewarti* take place during this stage of growth, the bacteria passing down the leaf through its vessels and entering the stem through the lower nodes. Natural size.

ECONOMIC ASPECTS.

The economic aspects may be considered under four heads: (1) Losses; (2) Natural methods of infection; (3) Conditions favoring the spread of the disease; (4) Methods of prevention.

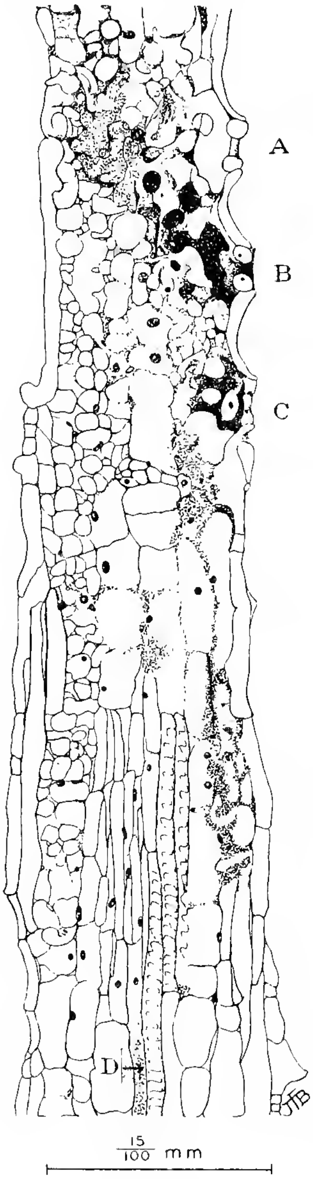

Fig. 74.*

In the United States Department of Agriculture and in our State Experiment Stations, naturally, much stress is laid on economic considerations, especially on 2, 3, and 4. A knowledge of 2 and 3 will frequently lead to some simple and effective means of prevention.

LOSSES.

It is desirable that there should be made from time to time a careful estimate of the losses caused by each particular disease, not only as a warning to farmers, fruit-growers, market-gardeners, and florists of the existence of these dangers, but also as an aid to legislatures and governments in deciding how much money may be judiciously appropriated for the scientific investigation of these problems. Pathologists are urged to make and publish such records. It is perhaps unnecessary to add that the determinations should be reasonably accurate, otherwise it were much better not to make any records. Occasionally, when diseases are widespread and destructive, so that depreciation of land values and the hostility of a community might result from great publicity, the pathologist may have to consider discretion the better part of valor and refrain from publishing, but in this event he should not fail to make full records which may subsequently be published or at least consulted. What we need and must finally have is a large body of accurate statistics, covering a series of years, many localities, and many diseases. To make these statistics most useful, certain meteorological data should be collected in the same localities. To be of most service this data concerning the weather should be recorded by the pathologist himself, who will be better able than anyone else to note down just those things likely to influence the host-plants favorably or unfavorably. Some of these things

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*Fig. 74.—*Bacterium Stewarti* (Erw. Sm.) attacking sweet corn (*Zea mays*). The section was cut from the extreme upper part of a seedling leaf which was fixed in strong alcohol six days after placing the bacteria on its tip. At the time of inoculation water was extruding from the leaf-tip, as shown in fig. 73. This figure represents a longitudinal vertical cut. The dotted and heavily shaded parts show the location of the bacteria which have entered through the ordinary stomata and have not yet penetrated the vascular system, although in places, as at D, they are close to the spiral vessels. At A, B, and C are three stomata. The substomatic chamber under A is free. B, with its surroundings, is shown more highly magnified in fig. 75. Drawn with help of the Abbe camera.

are cloudy weather (especially if prolonged), sunny weather, frequent or excessive fogs or dews, amount of rainfall, and frequency of rainfall, snowfall, hail, excessively hot weather, cold spells and frosts, droughts, daily maximum and minimum temperature, prevalence of special diseases correlated with special peculiar conditions, absence of other diseases, etc.

NATURAL METHODS OF INFECTION.

Under this heading the student should be on the watch for transmission of the disease through fungous or insect injuries, by mollusks, by birds or quadrupeds, and by the hand of man. Man contributes to the spread of diseases in various ways, *e.g.*, by neglect to remove diseased plants, by use of infected knives and other tools, by the introduction of infected seeds, or manures, or soils, or water, and by subjecting his plants to a variety of depressing and unwholesome conditions.

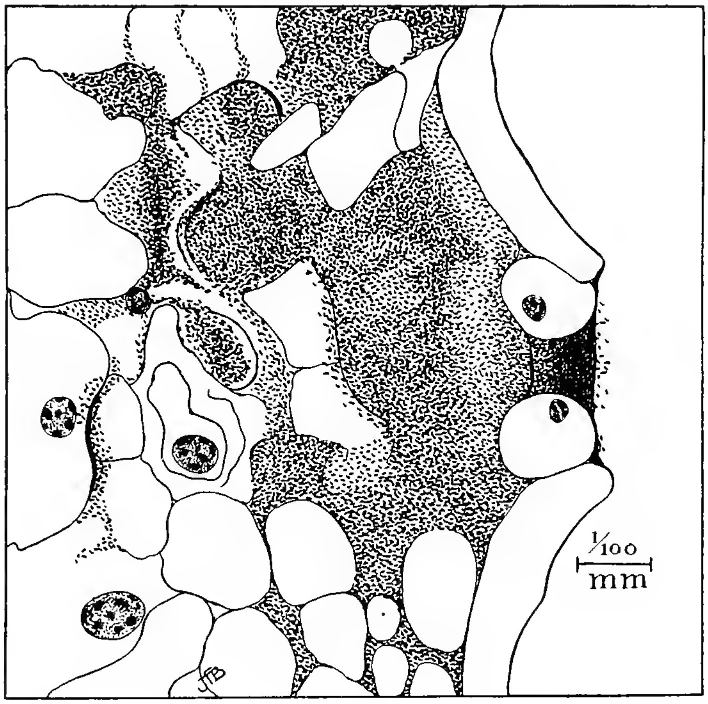

Fig. 75.*

A great variety of parasites find their home in the earth, the top crust of which swarms with bacteria and fungi. Such parasites are frequently introduced from one locality to another in infected soils adhering to wagons and other farm tools, to the feet of men and animals, to the roots of transported plants, etc. The soil is a living thing and it should not be transported even from one field to another on the same

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*FIG. 75.—*Bacterium Stewarti* filling the substomatic chamber and pushing out into the deeper tissues of a maize leaf. The result of an inoculation made by placing a small quantity of a pure culture on the tip of a sweet-corn leaf in the seedling stage. For orientation see fig. 74. The globose bodies are nuclei, which are not enlarged (?).

farm without due consideration of what may happen. Certain bacterial diseases might be distributed very readily in this way and good fields rendered worthless for certain crops.

The parasite may gain entrance to the plant through wounds (plates 2 and 4 and fig. 8) or by way of the stomata (figs. 70 to 75), lenticels, water-pores (figs. 76 to 79), and nectaries. In recent years the writer has discovered a number of very characteristic infections by way of the stomata and the water-pores, which are only modified stomata, *e. g.*, in cabbage, mustard, plum, bean, soy-bean, cotton (fig. 80), pelargonium, larkspur, broomcorn, sorghum, maize, cucumber, etc. Pear-blight affords one of the most striking examples of wholesale infection by way of the nectaries. The wilt of cucurbits affords an equally good example of infection through wounds—namely, leaf-injuries due to beetles.

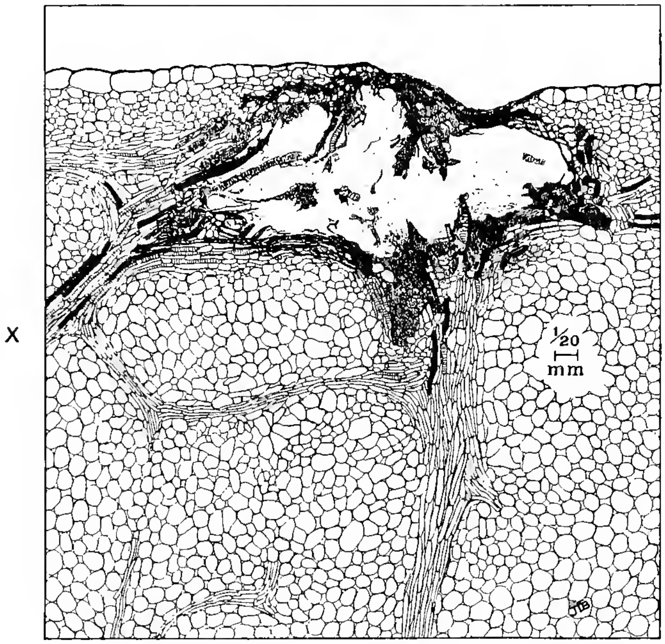

Fig. 76.*

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*Fig. 76.—*Bacterium campestre.* Section of a cabbage leaf parallel to the surface and near the margin, showing the result of infection through the water-pores. The tissues are browned and destroyed. Immediately under the leaf-serrature a cavity has formed and the bacteria have begun to penetrate into deeper parts of the leaf by way of the spiral vessels, not all of which are occupied. This figure is slightly diagrammatic, but only to the extent of omitting the protoplasmic contents of the parenchyma cells and of introducing six occupied spiral vessels which belong to the next section in the series. No spiral vessels are visible in the lower part of the section because the knife passed just below them. Material collected on Long Island, July 16, 1902, and fixed in strong alcohol. The spirals here shown are a little too densely occupied by the bacteria to make a good drawing under the oil-immersion objective, but a little farther in (beyond X) they are less abundant and entirely satisfactory for this purpose.

Conditions Favoring the Spread of the Disease.

The conditions favoring the spread of diseases may be wholly telluric, such as high temperature, unusual drought, cold weather, fogs, heavy dews, and excessive or continuous rainfall. These diseases may be favored by lack of natural drainage, or may be brought on by a variety of causes which are largely within the control of the grower, such as selection of improper varieties, *i. e.*, very susceptible ones, overcultivation, storage at too high temperatures (in case of cabbage and root crops), the use of infected soils, or manures, or seeds, or plants, and, especially in hot-houses, by the mismanagement of water and heat, and by the neglect to destroy the first diseased plants that appear and such transmitters of disease as insects and slugs, which frequently abound in hot-houses.

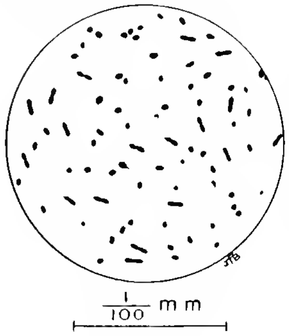

Fig. 77.[*]

Methods of Prevention.

In case of certain diseases, copper fungicides have been found useful, *e. g.*, in walnut bacteriosis and some of the leaf spots, but in general we know as yet very little about bactericidal treatments. In the early stages of an outbreak some of these diseases may be controlled by extirpation of the affected parts, or by the removal of whole plants as soon as they show signs. Also, if possible, the common carriers of infection should be eliminated. Finally, one should not forget that the substitution of resistant varieties for susceptible varieties is one of the most hopeful methods for disposing of certain of these vexatious diseases. Whenever anything specially noteworthy has been discovered in the way of treatment it will be mentioned under each particular disease.

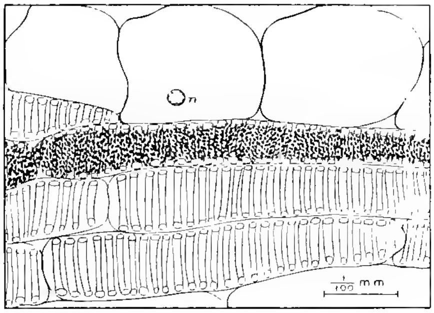

Fig. 78.[†]

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[*] Fig. 77.—*Bacterium campestre* from the cavity shown in fig. 76, illustrating water-pore infection of the cabbage. × 2,000.

[†] Fig. 78.—*Bacterium campestre* occupying a spiral vessel in a cabbage leaf near a group of infected water-pores. The tissues to the right and left of this vessel, and also above and below it (slide 223 a3, 18.6 by 9.7), are entirely free from bacteria. The body of the leaf and all its inner tissues up to within a few millimeters of the leaf-tooth, and also the outer surface of the leaf up to the water-pores, are sound. On the contrary, an unbroken bacterial occupation can be traced from this vessel outward to the water-pore region. The bacteria in this vessel are also less abundant than in those nearer to the group of water-pores, *i. e.*, its occupation is of more recent date. Even if there were no other evidence of infection by way of the hydatodes than that afforded by this vessel, the presence of the bacteria in it under the circumstances mentioned points conclusively to marginal (water-pore) infection as their only possible source. The position of this vessel is in a small vein a little below and at the left of **X** in fig. 76. Its distance from the left margin of the bacterial cavity is one field of the 16 mm. Zeiss objective with the 12 comp. ocular. Its distance from the sound leaf margin is two-thirds the diameter of such a field. A nucleus is shown at *n*.

GENERAL CONSIDERATIONS.

LOCATION OF THE LABORATORY.

If possible, the laboratory should be in a clean building in the middle of a green lawn. If it must be in a crowded and dirty city it should be on an upper floor, as far removed as possible from the dust of the street and from the tramp of feet. It ought not to be located on streets filled with the dust of heavy traffic. If a ground-floor or basement room in a dirty locality is the only available place, then the air which enters the room should be filtered through absorbent cotton. A south front is desirable for the mounting of a heliostat and for other photographic purposes; a north light is desirable for microscopic use, if one is to work at the instrument continuously. By arranging one's time according to the position of the sun, the light from east or west windows may be used to advantage five or six hours a day, which is quite long enough to fatigue ordinary eyes. The writer has managed to get along very well without north light for the last ten years. If one decides to use with the microscope only artificial light, such as that of the Welsbach burner, work-rooms for this purpose may be located anywhere. If possible, several rooms should be secured and apportioned to the various kinds of work, *e.g.*, general laboratory rooms, chambers for special workers, sterilization-room, thermostat-room, cold-storage and stock-culture rooms, storage rooms for chemicals, small glass-inclosed rooms for transfer of cultures, photographic rooms, dark rooms for developing, etc. The general photographic rooms should have overhead light as well as side light.

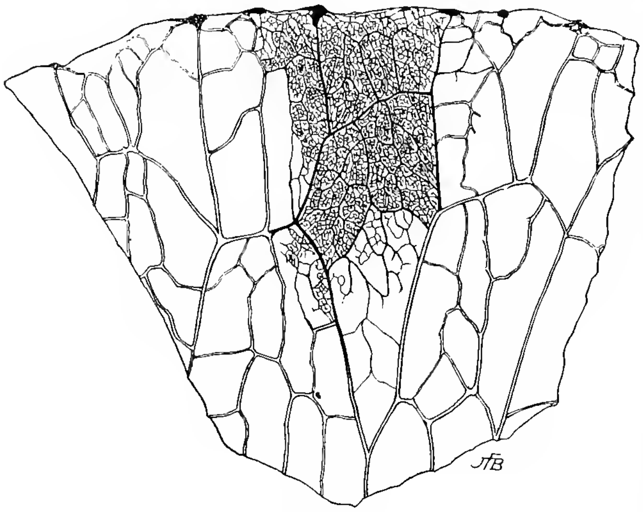

Fig. 79.*

EQUIPMENT OF THE LABORATORY.

Many pieces of apparatus may be procured from time to time, as the exigencies of the work demand or as the funds will permit. Other apparatus must be provided on the start, and some of it when the building is constructed or reconstructed.

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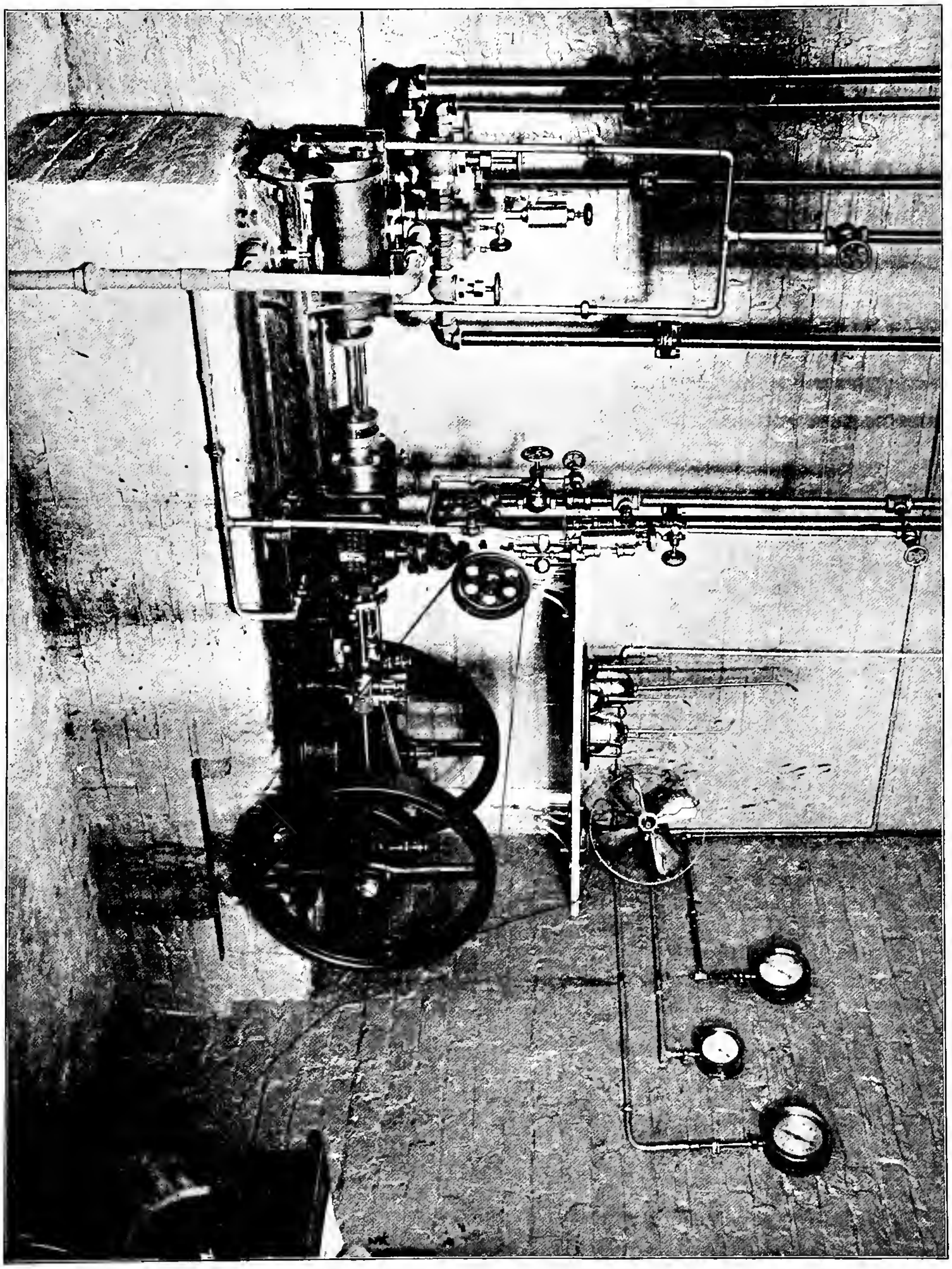
*FIG. 79.—Small portion of a cabbage leaf from Long Island, New York, showing characteristic water-pore infections due to *Bacterium campestre*. The blackened veins correspond to the location of the bulk of the bacteria which have gained entrance to the vascular system of the leaf by way of the groups of water-pores situated on the serratures of the leaf, particularly those which are conspicuously blackened. Those parts of the leaf where only the larger veins are shown were green and normal in appearance. Coll. July 16, 1902. Drawn from a photograph.



Engine-room, showing steam-pump for exhaust-air and compressed-air work.

Engine built to order by Guild & Garrison, Brooklyn, New York.

There should be hot-water pipes, cold-water pipes, steam pipes, a steam bath, gas-pipes, compressed-air pipes, exhaust-air pipes (plate 10 and fig. 81), and electrical wires for light and motive force. There should be thermostats, water-baths, cooled rooms, ice-boxes, steamers, dry-ovens, autoclaves, a distilled-water outfit, an alcohol-still (by which waste alcohol may be recovered or absolute alcohol prepared), an ether-still, filters, gas-generators, gas-furnaces, anaerobic apparatus, the very best microscopic outfits including apochromatic lenses, photographic and photomicrographic appliances, liquid-air receptacles, cylinders of compressed carbon dioxide and oxygen, microtomes, paraffin baths, glassware, balances, chemicals, and many minor pieces of apparatus.

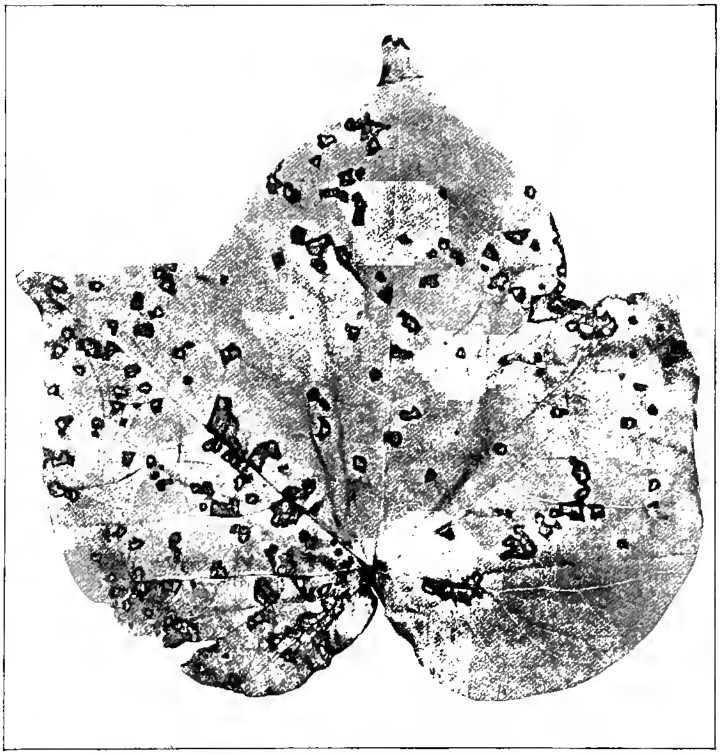


Fig. 80.*

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*Fig. 80.—Angular leaf-spot of cotton in which stomatal infections appear to be the rule. This leaf represents the secondary stage of a natural infection, *i. e.*, the spots have browned and shriveled, and they involve the entire thickness of the leaf. In an earlier stage of the disease the spots are limited to the under side of the leaf (mesophyll), and occur in the form of small water-soaked, uncollapsed areas surrounding stomata, under which nests of bacteria occur. These spots gradually deepen so as to involve the palisade tissue, and then they become visible on the upper surface of the leaf. The spots are not yet shriveled or browned, but if the leaf is held up and viewed by transmitted light they appear as translucent areas, while by reflected light they are dull and wet-looking. A little later they present the appearance shown in this figure. The writer has obtained all stages of this disease in Washington by spraying upon the plants young agar cultures of *Bacterium malvacearum* suspended in sterile water.

In general, the working capacity of a laboratory will be greatly increased by giving the director a stipulated sum of money per annum and carte blanche to buy laboratory necessities whenever and wherever and in whatever quantity he sees fit, requiring only that he submit vouchers; also by the employment of a number of subordinate assistants of special fitness, to whom may be assigned much of the purely mechanical and routine work of the laboratory, such as the proper cleaning of glassware, the making of ordinary culture media, the keeping alive of stock cultures, the preparation of staining media, the embedding, cutting, and staining of microtome sections, the making of photographs, the indexing of literature, etc. No scientific man should be willing to trust any piece of work in his own line to an assistant unless he can do it as well himself, or better, but when it has become to him the merest routine, his time, if worth anything, can be more profitably employed in something else. In most American laboratories which the writer has visited there is a woeful lack of intelligent subordinate assistance, such, for example, as that furnished by the German "Diener" and the Malays of Java. Every assistant can not hope to become at once an independent investigator, although, if competent, his work should always be shaped toward this desirable end.

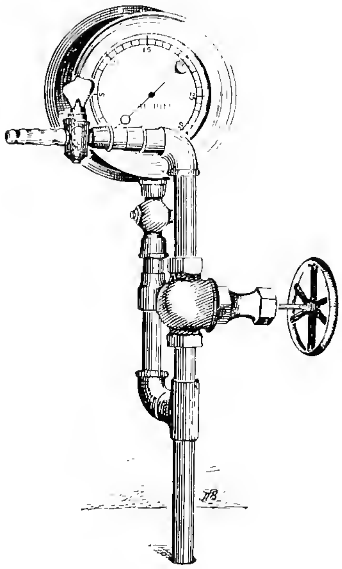

Fig. 81.*

A good library should be within easy reach, and as a suggestion to this end a list of useful books and papers is appended under the head of Bibliography of General Literature. A card catalogue of current literature is also very useful and in time becomes invaluable if properly made.

CARE OF THE LABORATORY.

The laboratory should be a clean place. Its walls should be of such material that they can be rinsed or wiped down occasionally. The floors, doors, tables, window-sashes, etc., should be wiped every day, every other day, or at least every third day, with clean cloths wet in distilled water, boiled water, or clean lake or artesian water. The use of river water, swarming as it does very frequently with all sorts of bacteria, is not to be commended for cleaning purposes, and brooms should be taboo. No one should enter the laboratory who has not business there, and order and quiet should prevail.

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*Fig. 81.—End of the vacuum-pipe on laboratory table. The gage serves to show the degree of exhaustion, *i. e.*, whether there is any leak in the piping between the engine-room and the laboratory. The two rooms should be connected by a speaking-tube.

PREPARATION AND CARE OF CULTURE MEDIA.

Everything should be carefully weighed or measured. Everything should be clean as possible to begin with. By water is usually meant distilled water, and this should be free from copper or other germicidal metals (see Bolton, Bibliog., XXXVIII). Moore & Kellerman have shown very recently that the *Bacillus typhosus* is destroyed in distilled water if the merest trace of metallic copper is present. Water swarming with this organism was sterilized simply by standing three hours in a copper vessel. The writer found the count of *Bacillus tracheiphilus* reduced over 30 per cent by exposure in bouillon in block-tin tubes for twenty-one hours. Exposure for forty-eight hours gave the same result, *i. e.*, 33 per cent destroyed. A simple glass still is shown in fig. 82. As far as possible the chemicals should be c. p., and in many cases it is necessary to make the test for oneself, no matter what the reputation of the firm or the statement on the label. When possible, broken packages should be avoided. It is therefore best to procure most chemicals in several small packages rather than in one large one. If the preparation of culture media is broken off before its completion, by nightfall or interruptions of any kind, the unsterilized or incompletely sterilized media should be put into the ice-box, especially if it is warm weather. Neglect of this precaution frequently results in the spoiling of the media. In steam sterilization one should begin to count time only after the thermometer registers 100° C., or at least 99° C. Those who live in high

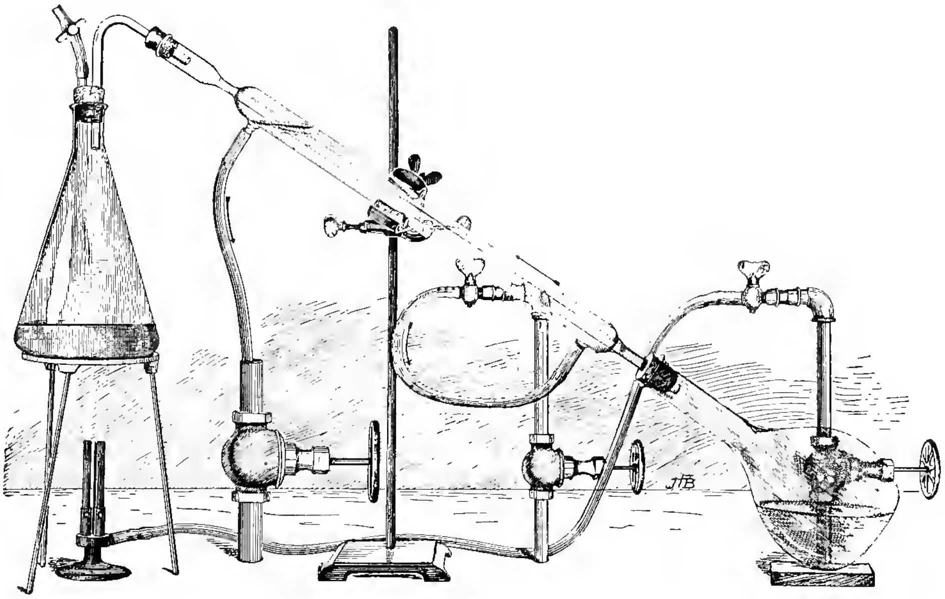

Fig. 82.*

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*Fig. 82.—Portion of a work-table showing method of distilling water for use in making culture media. The flasks should be insoluble glass. The cold hydrant water passes through the condenser in the direction of the arrow. In actual use the upright flask and the flame are sheltered from air-drafts by sheet asbestos. One-ninth actual size.

mountain regions must use autoclaves. Agar, potato, etc., in test-tubes, may be steamed twenty minutes on each of three consecutive days. Gelatin, beef-bouillon, and all other fluids likely to be injured by long heating should be steamed only ten or fifteen minutes on each of three consecutive days, if in tubes. The writer frequently steams such media fifteen minutes the first day, ten minutes the second, and five minutes the third. Agar, gelatin, bouillon, etc., stored in flasks in large quantity must be steamed a longer time—usually thirty to forty-five minutes on each day. The first steaming, when softened gelatin is added to bouillon, usually requires thirty minutes. To melt flasked agar quickly, shake it into fragments or break it with a sterile glass rod before putting it into the steamer.

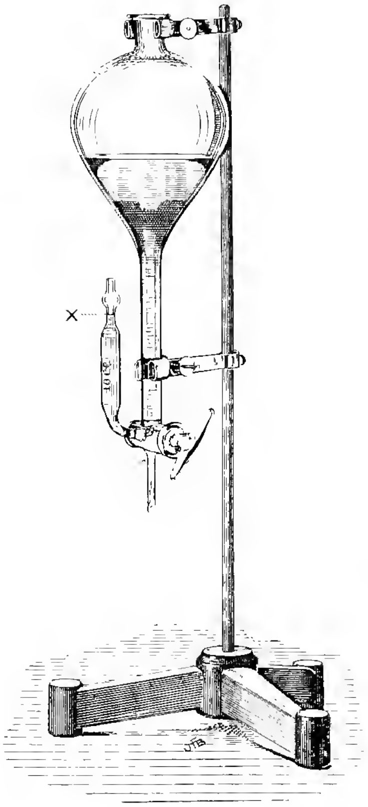

Fig. 83.*

Oversteaming should be carefully avoided. It softens gelatins or altogether prevents their solidification, and is very apt to cause troublesome precipitates in a variety of media. Precipitates in bouillon often occur if the tubes are not clean, or if the bouillon was not well boiled at first before filtering and placing in tubes. If the beef-broth looks greenish in the beaker or flask, rather than a clear yellow, it may be assumed that it needs more boiling and that if tubed in this condition it will throw down whitish particles on subsequent steaming. The writer prefers to obtain his ordinary + bouillons by incomplete neutralization with sodium hydrate rather than by addition of hydrochloric acid after full neutralization. The adding of hydrochloric acid precipitates out certain nutrient substances and also seems to interfere with the growth of some organisms. Distilled water and river water should be sterilized in quantity in the autoclave. For details concerning the making of particular media the student should consult the standard text-books, a dozen or more of which should be kept within easy reach in every laboratory. Some formulæ are given in the middle part of this volume. The autoclave may be used for the preparation of sterile water and some media, but, in general, I prefer media which has not been heated above 100° C., especially for use with sensitive organisms. Media should be heated in the autoclave only for a brief time and at a minimum pressure, generally not more than ten minutes and at not more than 110° C. Milk, gelatin, and media containing sugars should never be sterilized in the autoclave. Sugars and other substances decompose at these high temperatures and the results obtained by the growth of bacteria in such media are not comparable with those obtained on media sterilized at 100° C. Hitchens has recently shown that detrimental acids are formed when bouillon containing sugar is autoclaved. Peptone water, agar, and bouillon may be sterilized in the autoclave. For titrating culture media the writer uses the burettes shown in fig. 59. The twentieth-normal alkali is stored as shown in fig. 60. Quadruple-normal sodium hydrate solution is used for neutralization. The phenolphthalein solution is made by adding 1 gram of the dry powder to 100 cc. of 50 per cent alcohol, and then enough $\frac{N}{20}$ sodium hydrate to carry it fully into solution, removing the yellow color without making the fluid a very decided pink. Fluid media may be filled into tubes very rapidly by means of the device shown in fig. 83. For storing media sterilized in test-tubes and for holding cultures made on such media the writer has found ordinary quinine cans very useful (fig. 84).

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*Fig. 83.—Apparatus for rapidly filling test-tubes with 10 cc. portions of agar, bouillon, etc. By means of this device an expert assistant can fill 500 tubes an hour. Made to order by Emil Greiner. Height, 23 inches. The bulb above X is essential.

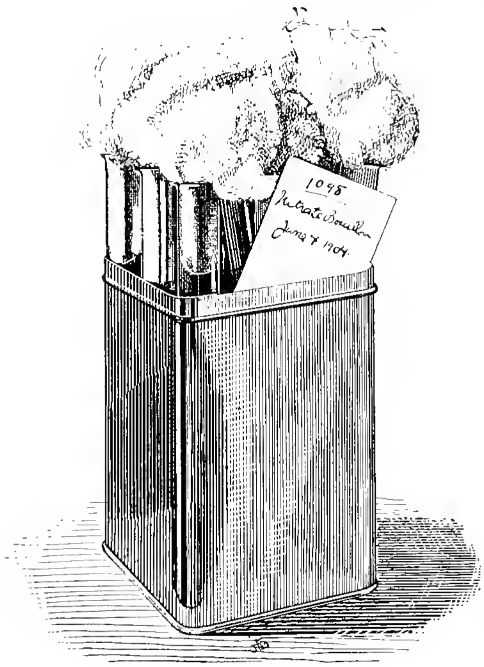

Fig. 84.*

The proper care of culture media after sterilization involves considerable thought if they are not to be used immediately. Stored media lose water and along with this loss, of course, there are physical changes, so that the results obtained are not always comparable with those obtained from similar media containing the standard volume of water. Various devices have been recommended for preventing this loss of water. Rubber caps keep in the moisture, but are apt to favor the development of fungi. Paraffined plugs made by removing the cotton plug, dipping the lower end of it quickly into and out of hot sterile paraffin, and replacing it in the mouth of the tube or flask before the melted paraffin has had time to cool, answer the purpose very well, but have the objection that all of the tubes must be placed in turpentine or some other solvent of paraffin before they can be cleaned for a second use. On the whole, the use of moderately tight plugs and the storage of the media in cool or cold air are the best methods of retaining the water content of the medium. Nutrient media should be made in small quantities and often, rather than in large quantities and at infrequent intervals. The cotton should be dry-heated in bulk before plugs are made from it.

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*Fig. 84.—Ordinary quinine cans with a little cotton in the bottom are very convenient for holding cultures and culture-media in test-tubes. One-third actual size.

THE CLEANING AND STERILIZATION OF GLASSWARE AND INSTRUMENTS.

New glassware may be boiled in soap-suds, rinsed thoroughly, soaked in the chromic-acid cleaning mixture for some hours, rinsed in hydrant water, soaked in several changes of distilled water, soaked or shaken in alcohol, and finally rinsed in distilled water. Neglect to wash in alcohol will frequently leave behind on the walls of the test-tubes an invisible film which causes vexatious precipitates in beef-bouillon, etc. Discarded tubes, flasks, and dishes containing living organisms must be autoclaved or filled with the chromic-acid cleaning mixture before they are washed. Some responsible person should attend to this. If acid is used it should be allowed to act for some hours.

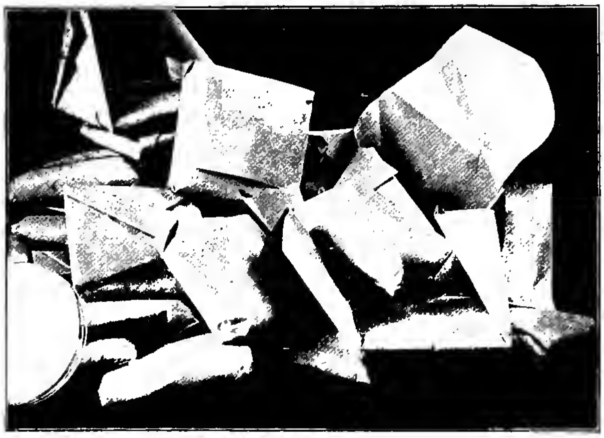

Fig. 85.*

Petri dishes should fit together well, but not tightly, and should be double-wrapped in clean Manila paper before placing them in the hot-air oven, or else should be inclosed in suitable tin boxes. The writer prefers to wrap them. The paper for this purpose may be 12 by 12 inches. The dish should be placed in the middle. The sides of the paper are folded over it; the corners of the projecting ends are then turned in, leaving V-shaped flaps, which are folded down on to the plate. The second covering is folded at right angles to the first and on the other side of the dish. Dishes treated in this way and ready for sterilization are shown in fig. 85. Pipettes should be dry-heated in the tin boxes already mentioned (fig. 37) after having the upper end carefully plugged with cotton, which should not project. Knives, scalpels, scrapers, spatulas, needles, forceps, etc., may be sterilized in the Bunsen flame, or, if needed cold in quantity, may be wrapped in Manila paper or put uncovered into short tin boxes and heated in the dry oven at 140° C. for two hours. Petri dishes, test-tubes, and all other apparatus wrapped in paper and put into the oven for sterilization by dry heat should have air spaces between them, *i. e.*, they should not be crowded together tightly, and the recording thermometer should project well down into their midst. The investigator should test the behavior of his oven when full and empty. Many cheap ovens give very different temperatures in different parts, especially if filled with apparatus, so that cotton or paper may be scorched in one part and not sterilized in another. The best oven known to the writer is that made by Lautenschläger. The improved form of the Lautenschläger oven shown in plate 6 does not require watching and gives a uniform temperature in all parts. It also furnishes a maximum temperature with a minimum consumption of gas, hot air being fed to the flame. The apparatus has an inner, outer, and middle wall. A horizontal iron gas pipe, of the relative size shown in the front of the picture, passes entirely around the apparatus at the bottom between the outer and middle wall. On top in this tube are many small openings through which gas escapes and when lighted forms so many small Bunsen flames. Air is drawn in at first and mixed with the gas in the middle open part of the feed pipe in front. The products of combustion escape through the chimney on top of the oven. There are pilot lights, so that the apparatus is set going easily. The result of this arrangement is that the middle wall becomes heated very hot, and consequently the air between this wall and the inner wall rises, cool air entering through holes in the bottom to take its place. There is thus created a powerful upward mount of hot air. This enters the oven through several hundred holes in its ceiling, is forced downward and escapes through as many holes in the floor. From this place the hot air is continually crowded sidewise and backward through brass tubes into the furnace chamber where it serves to support the combustion.

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*Fig. 85.—Petri dishes wrapped in two layers of Manila paper and ready to be dry sterilized. They are set on edge in the oven.

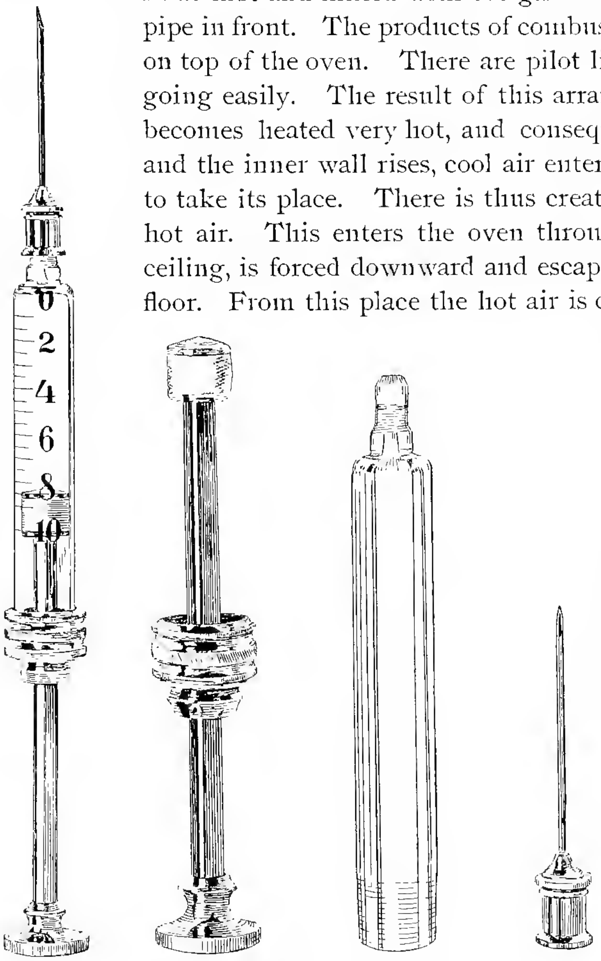

Fig. 86.*

Unless the dry-oven has a very uniform temperature throughout, so that there is no danger of scorching the cotton, plugged test-tubes should be tied together loosely and stood on end, cotton uppermost. Petri dishes (wrapped in paper as directed) may be set on edge. If the test-tubes have been properly cleaned, dry-heating is not necessary for such as are to hold steam-heated media, provided the cotton used for the plugs is dry-sterilized in advance. The best surgeon's absorbent cotton is not too good for this work. It should be unrolled and put into the dry-oven in a loose armful and heated just below the scorching point for several hours (2 to 3 hours at 145° C. will answer), with occasional unfoldings and turnings so that all parts may be heated uniformly. It is now taken out, re-rolled and put away in clean paper until needed. By this means all fungous spores lodged in it are destroyed and an oil is driven off which otherwise would be deposited as a whitish distillate on the inside of the test-tubes near the plugs. Hypodermic syringes may be sterilized by boiling in distilled water if the contaminating organism is non-sporiferous, or by soaking twenty-four hours in 5 per cent carbolic-acid water or lysol water and a subsequent soaking and boiling in pure water. The writer prefers the Meyer syringe, made by Lautenschläger (fig. 86). Syringes which allow the culture media to ooze out around the piston whenever any strong pressure is exerted are daugerous and should never be used with infectious material. Those which do not admit light or allow the experimenter to see how much fluid has been used or whether air is present are unsatisfactory. In case of many plants, needle-pricks are more satisfactory than hypodermic injections (pl. 4 and figs. 8 and 88). Needles are sterilized in the open flame as needed.

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*Fig. 86.—Dr. George Meyer's hypodermic syringe, made by Lautenschläger. Desirable on account of perfect workmanship, and because it is easily sterilized without injury. This size holds 1 cc. By twisting the button of the piston the packing at the other end is tightened or loosened at will. The separate parts are enlarged one-fourth.

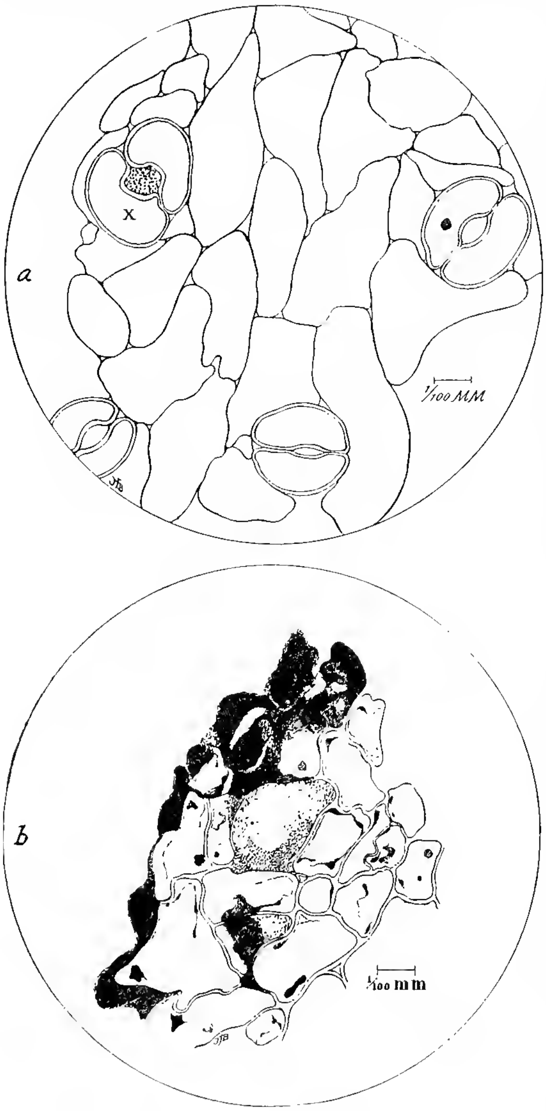

Fig. 87.*

When conveniences are not at hand, as on long trips in the country, the kitchen-oven may be used for sterilizing glassware, or even an open flame (alcohol lamp), and agar and gelatin for the making of poured plates may be melted by placing the tubes in hot water in a tin cup or tea kettle, but, in general, the writer has not found the rooms of ordinary farm houses very well suited for research work. Usually they are too dusty.

Surgeon's gauze is very convenient for laboratory use, for coarse filters, wipe-cloths, etc.

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*Fig. 87.—Early stage in the infection of a cabbage leaf by *Bacterium campestre; a*, epidermal layer on the apical part of the tooth of a leaf, showing one of the four stomata (X) full of bacteria. For the condition immediately under X see *b*, which was drawn from the third section in series, the intermediate one including part of the guard-cells. Slide 338, B1, stained with carbol-fuchsin. Drawn with the Abbe camera, 3 mm. Zeiss apochromatic objective and 12 compensating ocular. Material collected and fixed 8 days after infection, which was accomplished by atomizing upon the plant water containing a pure culture of *Bacterium campestre* grown on slant agar. When collected many of the serratures had begun to show traces of the brown stain which invariably appears when this organism grows in cabbage. The plant was inclosed in the cage shown in fig. 95, and was extruding fluid from its water-pores when it was sprayed. × 500.

THE MAKING AND TRANSFERENCE OF PURE CULTURES.

In addition to what has been said under *Pathogenesis*, the following suggestions may be of service to the beginner.

For the making of plate cultures and for the transfer of organisms from one culture medium to another, select a still day and, if possible, a day when a gentle rain or snow is falling. This offers ideal conditions, since the earth is wet, the outside air has been washed free from dust, and there is no wind to stir up dust within the laboratory. A strict adherence to this rule is sometimes very inconvenient and it is not meant to be iron-clad. It is, however, of immense service in keeping cultures free from contaminations, and those who propose to disregard it should remember that haste in the beginning of an experiment often leads to vexation and delay in the end, especially when the success of the experiment depends absolutely upon the purity of the culture.

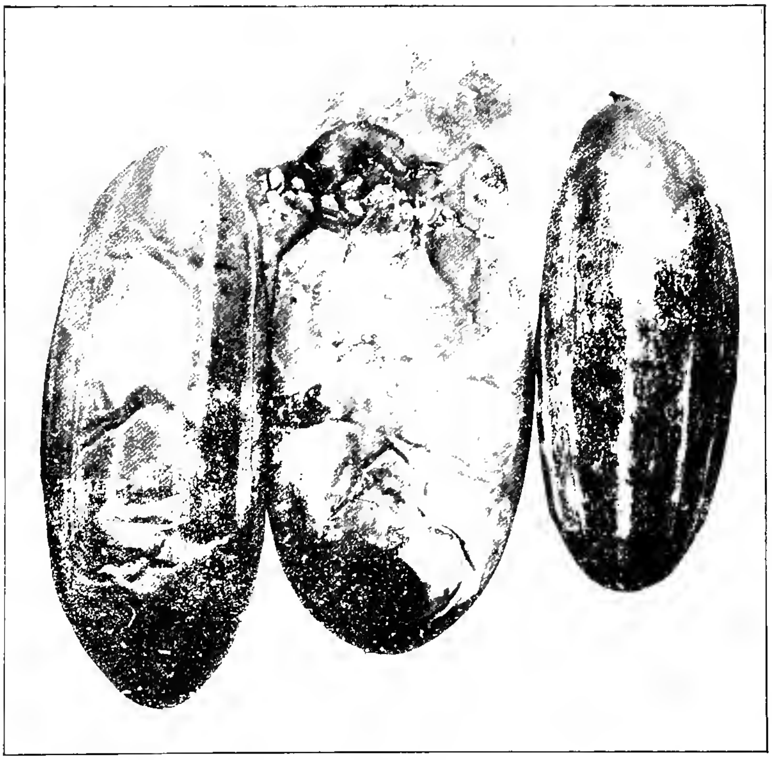

Fig. 88.*

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*Fig. 88.—Soft rot of green cucumbers inoculated by needle-punctures from a pure culture of *Bacillus carotovorus*. The only parts not softened are those through which the infected needle entered, *i. e.*, the parts rubbed with mercuric-chloride water. In each a little button of tissue under the disinfected area did not decay. The sound fruit at the right was punctured at the same time, but with a sterile needle. The cucumbers had been removed from the vine, but were not flabby. They were exposed after inoculation to the ordinary air of the laboratory. The photograph was made on the seventh day. About two-fifths natural size.

When ready to make the transfers or to pour the plates, close the windows, wipe up the tables, and wet down the floor, window-sashes, etc., with distilled water or boiled water, and reduce the air-currents within the laboratory to a minimum (especially when transfers are to be made in the open room) by keeping the doors shut and restricting the movements of all persons who may be in the room. It is much better to do all of this work in specially constructed small rooms (plate 11) than under hoods (plate 12). Hoods are open only in front. They may be made of any convenient size. The one here figured is is 32 by 39 by 20½ inches, outside measurements. When one is far from laboratories small hoods may be extemporized out of clean paper, or cultures may be poured and transfers made inside of a clean pail or jar, turned down on its side. Any method, in fact, which restricts the movement of air past open plates and tubes will be found serviceable.

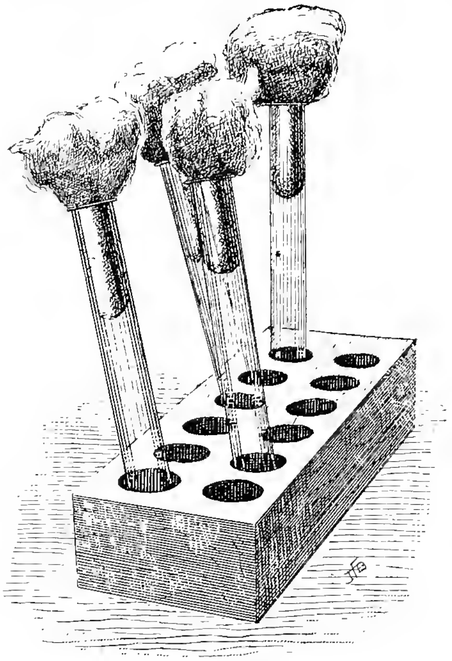
Fig. 89.*

The work-shelf of the room shown in plate 11 faces a window as wide as the room, and extending from the level of the shelf to the height of the other windows in the room. This window faces south and is only 6 feet from a well-lighted window in the outer wall of the building. The room also receives bright light from the west side. At the front end of the shelf are a Bunsen burner with cut-off flame, a box of safety matches, a box of rubber bands, and two tumblers—one for burned matches and one for platinum loops, needles, forceps, etc. Immediately under this part is a narrow drawer for pencils, note paper, knives, etc. At the back end are a few wrapped Petri dishes, a nivellation apparatus, a flask of sterile water, and a crate of media. Underneath this part is a second shelf 3 inches below the first, where Petri dishes and tubes containing solid media may be put out of the light as fast as inoculated. The size of this room (inside measurement) is 4 by 4 by 10 feet, and it is large enough. No provision is made for ventilation, because air-currents in a culture-room are very objectionable. The windows, walls, and floor are wiped up with distilled water before making transfers. Outside is a bit of the author's private laboratory. At the right is the microtome and behind it on the wall are deep and shallow drawers; 69 is for bulk paraffin; 70 A, B, C, D, E, are cut into small compartments used for paraffin blocks. The very shallow drawers are for ribbons which can not be mounted the day they are cut; 72 has a series of shelves opening on the south side and is used to hold photographic printing frames.

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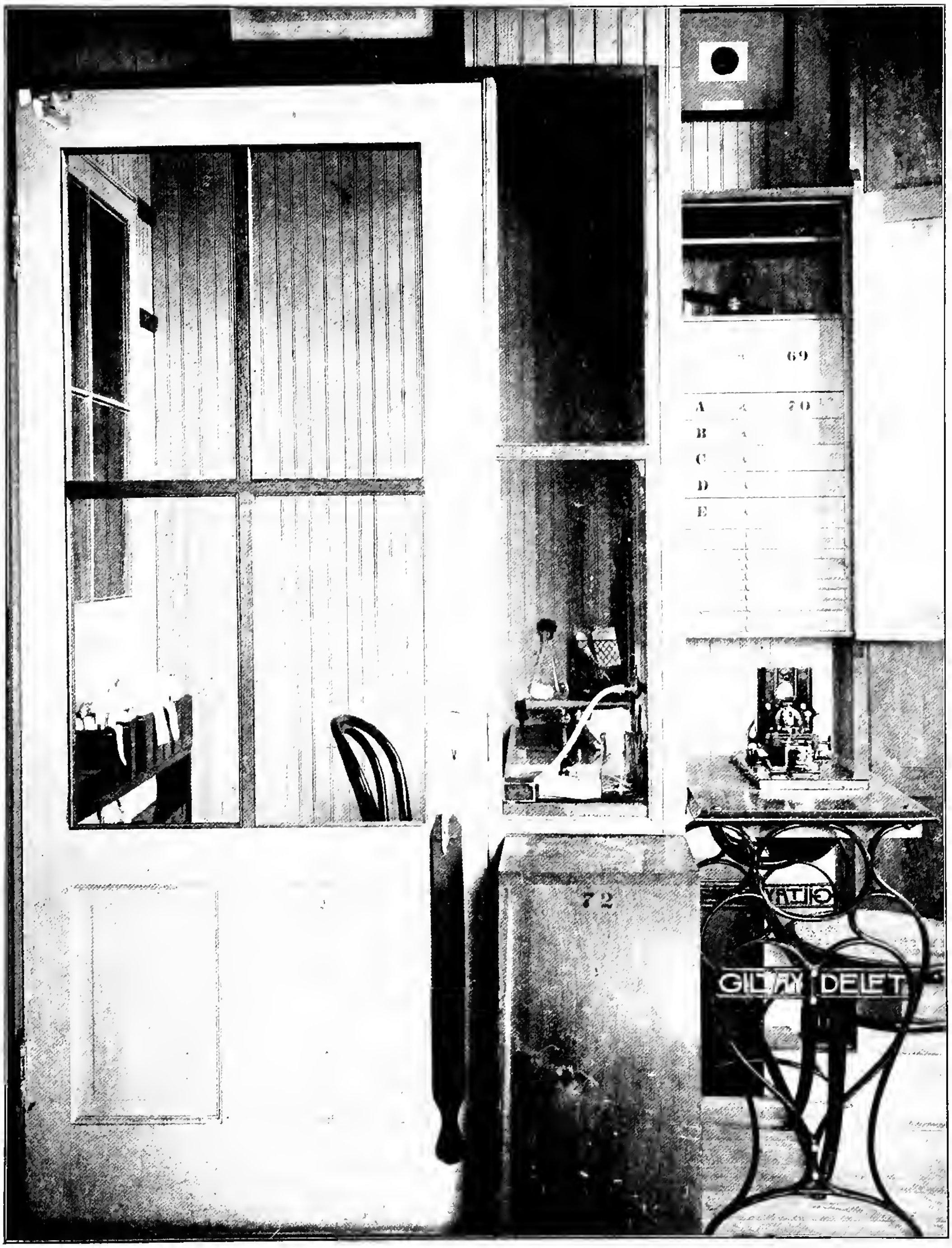
*Fig. 89—Pine block with inch holes, convenient for holding test-tube cultures during examination, or tubes of media which are to be inoculated. A good size is 9½ by 3½ by 1⅞ inches.



The author's culture-room.

At the left hand (back) are narrow shelves for culture-media, pipette-boxes, etc. At the right is the work-shelf, covered with plate glass.

The agar may be poured at 42° C. in case of organisms whose thermal death-point is known to be high (50° C. or above). For all others it must be cooled carefully to 40° C. before inoculating for poured plates. This requires five or six minutes in the water bath at 40° C. Even this temperature is too high for some organisms and then gelatin at 30° C. may be used. When ready to pour, take a clean absorbent cloth and carefully wipe all water from the outside of the tube (the lips of which have been previously flamed *gently* with a rotation of the tube on its long axis), lift the cover of the dish only as much as is necessary, hold the cover *over* the dish (not at one side), pour quickly but gently, and re-cover, tilting the dish about quickly but gently, if the fluid has not already covered the bottom. To entirely cover the bottom sometimes requires a smart little jerk, if the agar is not very fluid. The student must learn to work rapidly and dextrously, then there will be no complaint that the agar has solidified before the plates are poured. The plates should be set on a level shelf while the agar or gelatin is hardening, or, if the colonies per square centimeter are to be determined, a nivelling apparatus such as that shown in fig. 66 must be used, and the dishes should have flat bottoms. When plates have been inoculated too abundantly to secure subcultures from single colonies, these may sometimes be obtained from the traces of agar or gelatin left in the tubes from which the plates were poured. With this end in view, these tubes should be re-plugged and laid away, for a few days, the lips and top of the tube which were wet by the agar or gelatin being first heated hot in the flame, care being exercised not to crack the tubes.

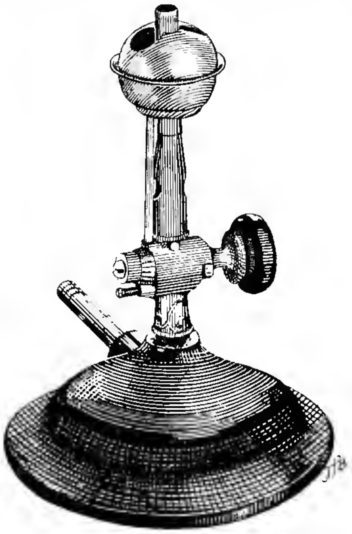


Fig. 90.*

All tubes containing fluids should be opened and inoculated in a position as nearly horizontal as their contents will permit, and tubes of solid media, such as agar, may be held level or inverted for inoculation. A convenient block for holding test-tube cultures during examination is shown in fig. 89. It is usually best to flame the plugs slightly before their removal, particularly if they have been exposed to the air for some days. As an additional precaution the transfers should be made under a glass hood, or in a special culture-chamber. If sterilized needles, loops, knives, forceps, pipettes, or anything else designed to be used in making the transfers have accidentally touched *anything whatsoever*, they are presumably contaminated and must be rejected or reflamed. Do not handle the lips of test-tubes containing gelatin or agar from which plates are to be poured. Your hands may be contaminated by resistant spores. Take hold of the tubes lower down. To economize gas and avoid heating the air of the small work-chamber to an uncomfortable degree, small, cut-off, constant-flame burners are very convenient (fig. 90).

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*FIG. 90.—A constant Bunsen burner with cut-off flame. Very useful for the laboratory table and the culture room. About two-fifths actual size.

Plates, tubes, and flasks containing pure cultures or designed for inoculation should *never* be opened in the general laboratory on a windy day or in air currents. Pour two uninoculated agar or gelatin plates in the proper way. Keep one covered and uncover the other for a few moments in a current of air, *i. e.*, as long as the time required to make a plate culture. Then keep the two plates together and compare from time to time. A few experiments of this sort will convince the most skeptical of the necessity of avoiding drafts.

The person and clothing of the experimenter should be as clean and free from dust as possible. White duck coats are very convenient. They show at once when they are soiled and need washing and ironing.

Organisms which for some reason may be difficult to obtain in ordinary plate cultures and which differ markedly from their associates in some particular way, *e. g.*, by more rapid growth, by indifference to heat, to acids, to thymol, to chloroform, to absence of air, etc., or which can use, as food, substances which will not support the growth of most bacteria, may sometimes be isolated very readily by providing conditions suited to their growth and unsuited to that of the bacteria with which they are mixed. This is Winogradsky's principle of *elective culture*. As he defines it, this is a culture "which presents conditions favorable only to a single definite function or, more exactly, to a function as strictly limited as possible." Such media or methods are exactly the opposite of universal. Nutrient starch jelly and nutrient silica jelly are good examples or such media. Nutrient fluids rich in acid potassium phosphate or destitute of nitrogen are additional examples.

Fig. 91.*

Heat is often an excellent means of separation. Winogradsky separated his *Clostridium pasteurianum* from all but two of the contaminating species by heating ten minutes at 75° C. (Archives des Sci. Biol., Vol. III, p. 310). The isolation of *Streptococcus* (*Leuconostoc*) *mesenterioides* by Liesenberg & Zopf and of *Bacillus hortulanus* by Sturgis are other examples of separation by heat. Omélianski's separation of his hydrogen-cellulose ferment from his methane-cellulose ferment by exposure of the recently established methane ferment to 75° C. for fifteen minutes is another good example.

THE FINAL DISPOSAL OF INFECTIOUS MATERIAL.

Diseased material should not be left around the laboratory any longer than is necessary. When it has served its immediate purpose that which is not to be preserved permanently should be thrown into the furnace. Small amounts may be sterilized by putting into beakers or jars and covering with cleaning mixture or equal parts of crude sulphuric acid and water. Crude vegetable and animal sub-

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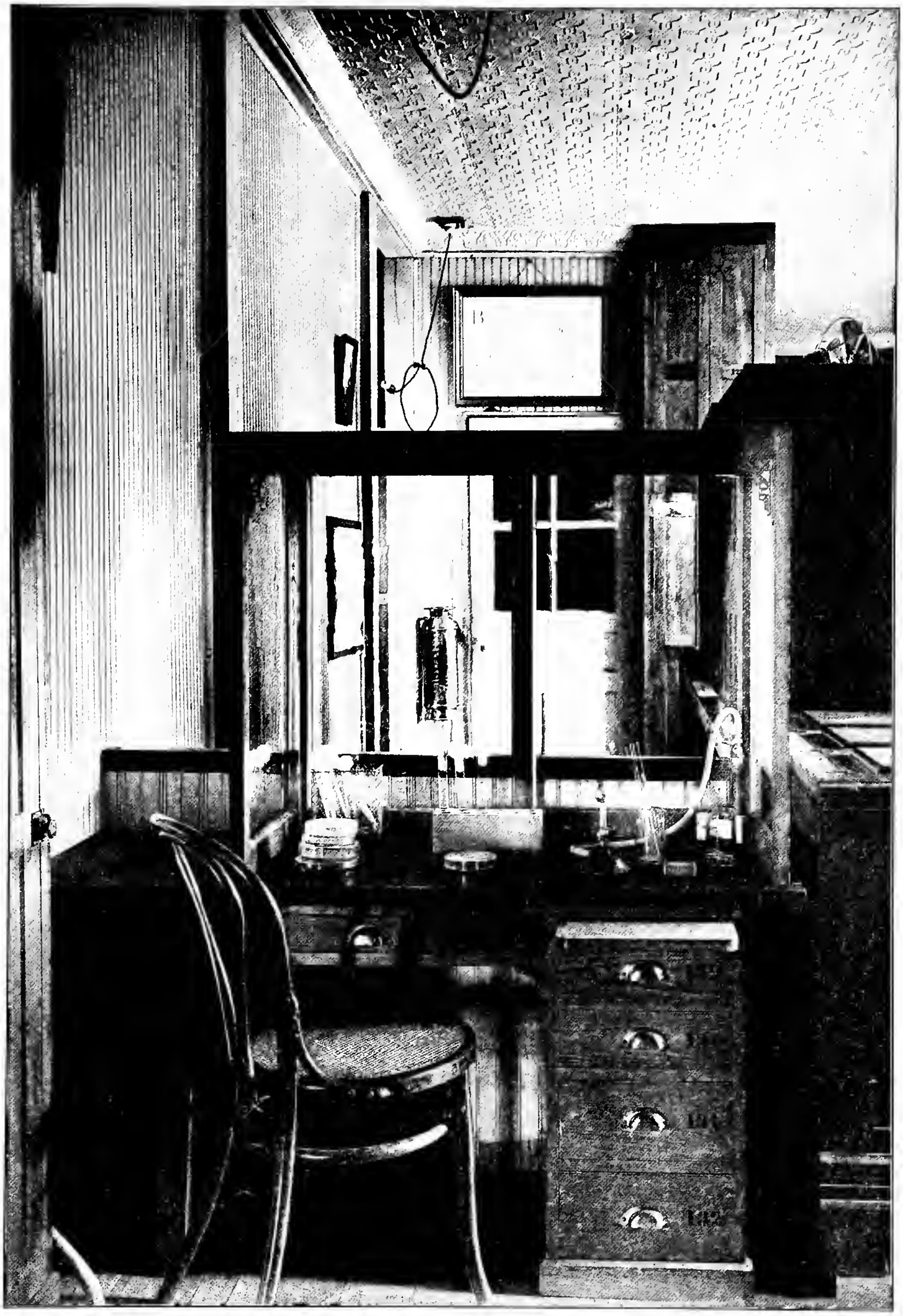
*Fig. 91.—Instrument for making puncture-inoculations. It consists of a bone handle with a metal-screw socket, into which a sewing needle is thrust. The needle is usually of small size—a No. 8 or 10.



Work-table with movable frame of wood and glass.

Bacteriological transfers may be made under this frame in the open room if windows and doors are kept closed.

stances likely to become moldy must never be stored in refrigerators designed for pure cultures. The open ice-box is the proper place for such substances, and they must not be left there indefinitely. Some people have a mania for collecting everything and then keeping it a long time without making any use of it. An ice-box treated in this way soon becomes an intolerable nuisance.

Discarded plates, tubes, slides, covers, pipettes, contaminated litmus paper, etc., should be autoclaved, or covered or filled with cleaning mixture, or dropped into it, as the case may be. Deep, narrow glass jars or long, rectangular enameled pans are necessary for the pipettes. Soiled hands may be disinfected with mercuric-chloride water (1:1000), which should always be on hand in the laboratory in quantity properly labeled. Slight wounds should be washed five or ten minutes in this fluid. Surfaces of floors, tables, etc. soiled by spilled bacterial cultures should be covered immediately with mercuric-chloride water (1:1000) and wiped up carefully after ten or fifteen minutes with distilled water. Spilled cultures of molds should be soaked in mercuric chloride (1:1000) for at least an hour before wiping up. Neglect of these simple rules means the seeding down of the ice-boxes, culture-chambers, and the general laboratory with all sorts of resistant mold spores and bacteria. An abundance of cheap carbonate of lime should be kept on hand for the prompt neutralization of spilled acids. A mass of cotton waste is convenient for the prompt mopping up of spilled fluids.

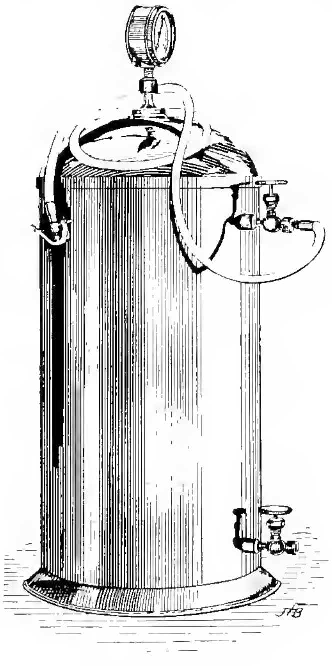


Fig. 92.*

All contaminated needles, loops, knives, scissors, forceps, etc., may be sterilized in the open flame. Instruments which are too valuable to be flamed may be sterilized in carbolic acid (5 per cent) or formaldehyd (5 per cent) or lysol (5 per cent). *Never* put down a platinum needle or loop which has been used in making transfers until it has been passed carefully *its whole length* through the flame. Dissections are best made on trays which can be easily cleaned and sterilized.

---

*Fig. 92.—Compressed-air tank and spray-tube. The one here shown, made by Boeckel, Philadelphia, is nickel-plated and very substantially constructed. It is filled by means of a small pump similar to a bicycle pump. The gage registers up to 100 pounds per square inch, but 40 pounds pressure is ample. The bacterial fluid is placed in atomizers of the form shown in fig. 93. The method of attachment is not satisfactory. This device is very convenient when trees or low plants covering a considerable area are to be inoculated. Height, 20 inches. The same firm has devised a compact traveling outfit, the compressed-air tank being about one-half the size of the one here figured. The whole is packed into a neat portable box, and the only disadvantage is the small size of the air-chamber, which requires more frequent pumpings. Of course the apparatus may be used equally well for the distribution of fluid germicides or insecticides.

METHODS OF INOCULATION.

Inoculations may be by punctures with a delicate needle (fig. 91), by abrasions of the surface, by hypodermic injection, by watering the soil with infective material, by plunging aerial parts into infectious liquids for a longer or shorter time, by simply putting the bacteria into drops of water on parts of the plant and protecting from sunlight and evaporation for some hours, or on a larger scale by spraying portions of the surface with very dilute culture fluids or, preferably, with water containing the bacteria (figs. 92, 93, 94), by brushing or rubbing cultures into some part of the surface, by allowing insects, snails, etc., to feed on diseased material and then colonizing them on healthy plants. The writer has made good use of this last method in case of three different bacterial diseases. Stomatal infections may be secured by subjecting the plants to conditions similar to those occurring in nature on dewy nights or during heavy fogs or prolonged rains, *i. e.*, by placing the potted plants on wet sand, atomizing thoroughly with sterile water and covering with tall, roomy bell-jars. The experiment should be undertaken in a cool rather than a warm house. When the right conditions have been obtained, moisture covers the surface of the plant in tiny drops which do not evaporate. The bell-jar may now be raised and the plant again atomized lightly with sterilized water containing the bacterium. The best time to do this is late in the afternoon, so as to take advantage of the cooler night temperature. When the bell-jar is returned, which should be immediately after spraying, it should be covered with cloth or paper to protect from the light. Usually bell-jars should be removed at the end of twenty-four hours, but exceptionally they may be left on thirty-six to forty-eight hours, if not exposed to the sun. Inoculation cages are very convenient for small plants (fig. 95). In case of trees, or shrubs, or masses of tall herbs, tight-fitting covers of tent-cloth will be found serviceable for obtaining conditions similar to those prevailing in wet weather. They may be left on 1 to 3 days, the outside of the tent as well as the plants within being sprayed with water often enough to keep everything moist until infections have been secured.

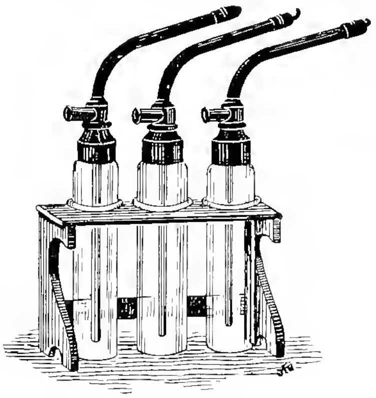


Fig. 93.*

When the nature of the plant will permit it and when only a few inoculations are to be made, the surface which is to be punctured should be rubbed thoroughly for three to five minutes with mercuric-chloride water (1:1000) and then washed with equal care in sterile distilled water. When many inoculations are made with large numbers of check plants and when due care has been taken to work under conditions such that accidental contaminations from the same organisms are not to be feared, the writer has not found this precaution necessary. The use of mercuric chloride should be avoided, if possible, especially on leaves, as the writer's experiments have shown that it penetrates into the plant (some plants) for a considerable distance and prevents the action of the bacteria to this extent (fig. 88), if not altogether, as has happened in some cases.

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*Fig. 93.—Atomizers for use with the air-tank (fig. 92). These are made by the Davidson Rubber Company, Boston, Mass. About one-fourth actual size. The De Vilbiss sprayer, made in Toledo, Ohio, and now used by the writer, has several distinct advantages. It is all metal and can be sterilized in boiling water without becoming twisted out of shape, it can be attached more easily to large flasks and to the tube leading from the compressed-air tank, and the spray may be directed up, down, or straight ahead without changing nozzles. It requires, however, more force to operate than the Davidson sprayers, and consequently is less convenient when used with a hand-bulb.

THE KEEPING OF RECORDS.

If one contemplates doing much work, a careful record of what has been done is as important as the experiment itself, since exact remembrance is certain to pass away with lapse of time.

In all his work, the student should accustom himself to make very exact statements, so that others may be able to follow him. For example, he should not describe his organism as "yellow" or "red" without qualifications, since there are many yellows and reds, but should carefully compare it with some standard color-scale (Ridgway's, Saccardo's, Standard Dictionary, etc.), and govern himself accordingly. He should not say, "Organism does not grow at room-temperatures," but rather should state the temperature at which growth does not occur, as 15°, 25°, or 35° C., any one of which may be "room-temperature," depending on the latitude, altitude, and time of year. He should not say, "Organism is killed at temperature of 65° C.," without at the same time stating the age of the culture, conditions of exposure, and time required, which might be ten days or five minutes.

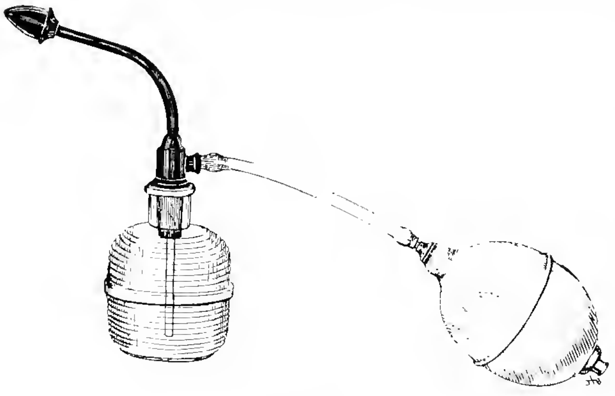

Fig. 94.*

Every independent worker will in the end devise a method of note-taking which is more or less characteristic of his personal peculiarities and best adapted to his own particular needs. For all persons there is no *one best* method. The methods described in the following paragraphs have been settled upon as those most convenient for the writer, but it does not follow that they are the most economical of time, or the best devisable, or the ones which independent workers should follow. They are here given as hints for beginners and because the method a man employs in his work is always a matter of more or less interest to his fellow-workers.

First of all, there should be provided a record book in which the method of preparation of each culture medium is carefully described. This should be a good-sized book, well bound in leather, so as to stand long and hard usage. The entire quantity of a culture medium is known as a "stock" and receives a special number, which is written, pasted, or stamped on any flask or tube that contains it and which serves to identify it. If a stock is subsequently divided and a portion of it is treated in some different way, *e. g.*, receives more sugar, acid, or alkali, this portion receives a new number, or the old number with the addition of a letter of the alphabet. Each stock described in the record book is numbered serially from 1, and the book continues in daily use as long as the laboratory, or until it is filled with records and carefully filed away as "Culture Media, Volume I."

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*Fig. 94.—Hand-sprayer which may be used for distributing bacteria on plants. Some form is usually kept in every pharmacy and sold as a cologne atomizer.

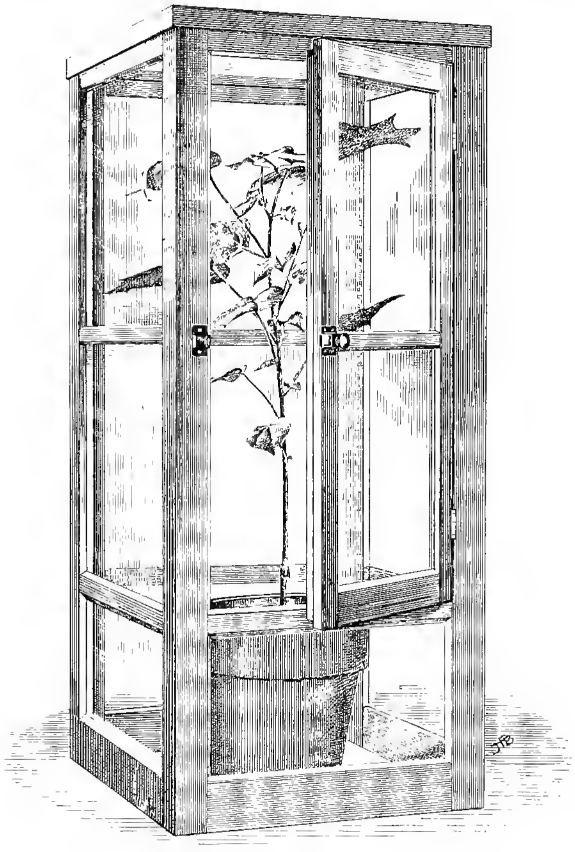

Fig. 95.*

The small pocket ledger, No. 492 of A. C. McClurg & Co., Chicago, is very convenient for certain kinds of notes, especially those made in the field and those required for the identification of alcoholic specimens and stained slides (fig. 112). All records should be in ink, of a sort which does not fade, and in field work a *good* fountain pen is invaluable. Pencil records, especially those made with rapid-writing soft pencils, soon become illegible and should not be tolerated except on paper to be subjected to steam heat.

Large sheets of well-gummed paper should be procured and the labels cut in the laboratory to the size needed. Labels may be cut rapidly in quantity with the apparatus used to trim photographic prints for mounts. When exposed to streaming steam such labels come off easily, and it is best not to paste them on the tubes or flasks until after the final steam sterilization. In moist climates, stock quantities of such gummed labels must be kept in air-tight boxes or between sheets of paraffined paper. Test-tubes in crates are kept separate during steaming by writing the number of the stock on a slip of paper and thrusting this into the crate with the test-tubes. The number should be written with a lead pencil. Faber's pencils for writing on glass are useful in case of flasks and fermentation tubes, since records made with these pencils will bear streaming steam. An inexpensive black pencil which writes on clean glass very readily and bears steam well (even better than Faber's) may be made by stirring into melted beeswax enough lamp-black to make a thick-flowing liquid (as thick as will flow). This is poured into molds made by wrapping writing paper, in several turns, around a lead pencil or thick glass rod, tying near one end, removing the rod, squeezing the other end flat, turning over its edge, and fastening this flattened end in a split stick or clamp. The paper should be retained as a cover, the string being removed and the loose edge pasted down. A dozen such pencils may be made at a cost of 10 cents. In the absence of such pencils, flasks and fermentation tubes may be distinguished in the steamer by dropping over the neck different-sized rubber bands or different numbers of the same kind of band, or by writing with a lead pencil the number of the stock on a square of letter paper, cutting a hole in its center and slipping this over the neck of the flask or tube. When the steaming is over, the regular labels should be pasted on or the stock number written on with the proper pencil.

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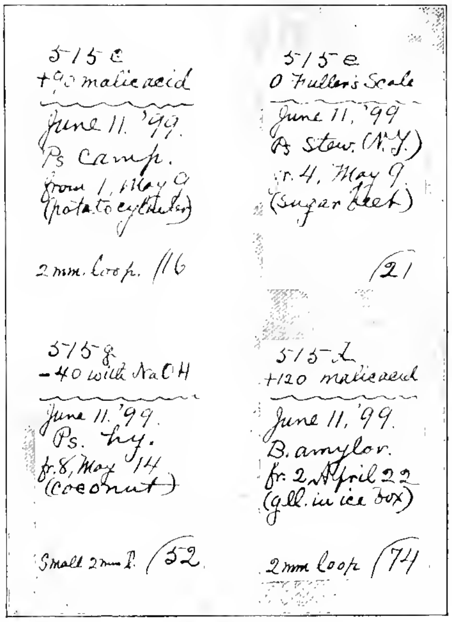
*Fig. 95.—Small cage of wood and glass in which herbaceous plants may be placed for inoculation by spraying. The inside measurements are 12 by 12 by 30 inches. The large door is a great convenience. Hook-fastenings are better than spring catches.



5/15 e
+90 malic acid
June 11. '99.
Ps camp.
from 1, May 9
(potato cylinder)
2 mm. loop. /16

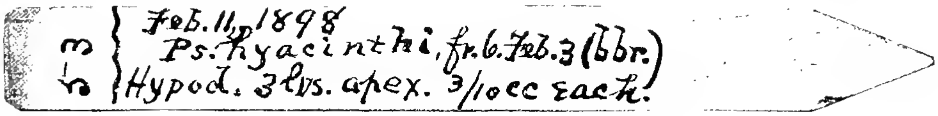
5/15 e
0 Fuller's Scale
June 11, '99
Ps Stew. (N.Y.)
fr. 4, May 9
(Sugar beet)
/21

5/15 g
−40 with NaOH
June 11. '99.
Ps. hy.
fr. 8, May 14
(cocoanut)
Small 2 mm l. /52

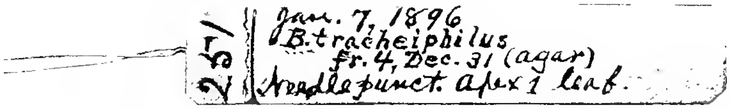
5/15 L
+120 malic acid
June 11, '99.
B. amylov.
fr. 2 April 22
(gel. in ice box)
2 mm loop /74

Fig. 96.*

All plate cultures and all subcultures made on a given day, no matter of what organism, are numbered serially, beginning with 1. These are 1, 2, 3, etc., of that particular day. Those of any other day are also numbered 1, 2, 3, etc. The writer usually numbers his plates I, II, III, etc. Labels may be pasted on the covers of the Petri dishes, or all may be done with the glass pencil. Cultures in tubes subject to frequent handling and likely to be needed for some time should have gummed-paper labels written in ink. The above transcripts from labels on four test-tube cultures



Feb. 11, 1898
Ps. hyacinthi, fr. 6. Feb. 3 (bbr.)
Hypod. 3 lvs. apex. 3/10 cc each.



Jan. 7, 1896
B. tracheiphilus
fr. 4, Dec. 31 (agar)
Needle punct. apex 1 leaf.

Fig. 97.*

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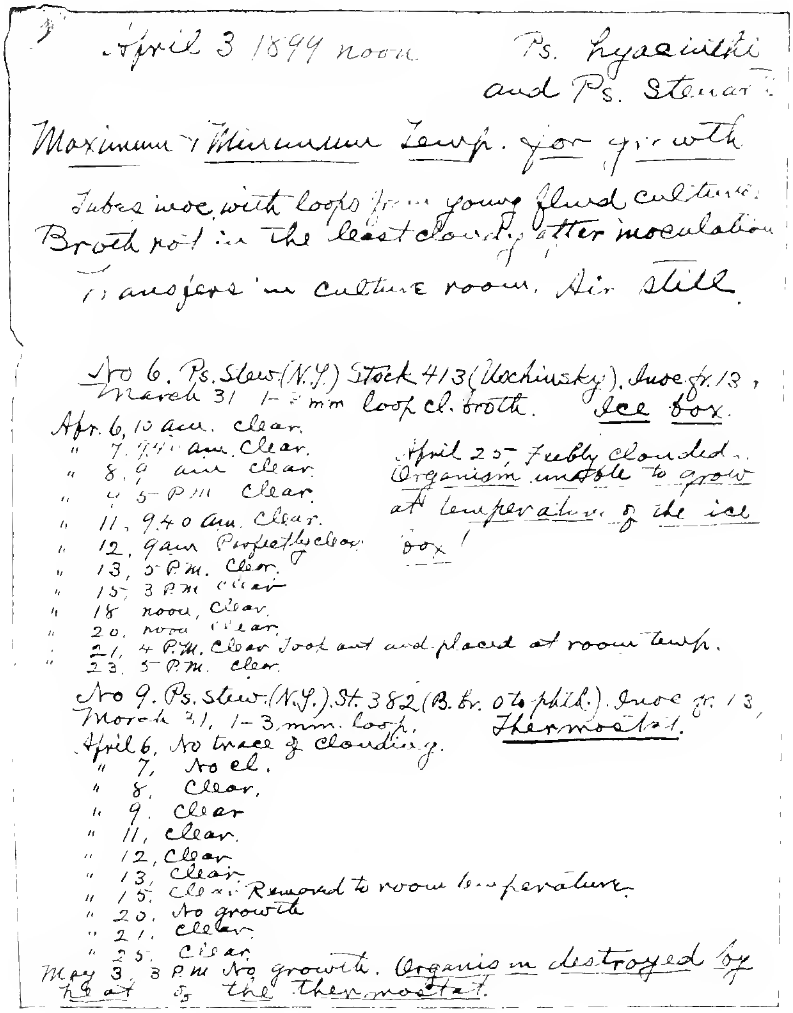
*Fig. 96.—Labels from test-tube cultures.

*Fig. 97.—Wooden labels from inoculated plants.

(fig. 96) sufficiently indicate what is necessary to form a satisfactory record. This could, of course, be considerably abbreviated by a system of symbols or by depending to a larger extent on the "Notes."

In case of the inoculations, on the contrary, only as many series are made use of as there are diseases under consideration. Each plant is generally given a single number, no matter in how many places it may be inoculated, the separate inoculations being kept distinct, if necessary, by sub-numbers. Each series begins with No. 1 and continues in an unbroken sequence as long as the disease is under consideration. The labels written on soft wood, covered for this purpose on one side with white paint, are stuck into the earth or wired to the plant. Transcripts from two such labels are shown in fig. 97.



April 3 1899 noon. Ps. hyacinthi and Ps. Stewarti.

Maximum & Minimum Temp. for growth

Tubes inoc with loops from young fluid cultures.
Broth not in the least cloudy after inoculation
Transfers in culture room. Air still.

No 6. Ps. Stew. (N.Y.) Stock 413 (Uschinsky). Inoc fr. 13, March 31 1-2 mm loop cl. broth. Ice box.
Apr. 6, 10 am. clear.
" 7, 9.40 am. Clear.
" 8, 9 am clear.
" 9, 5 P.M. Clear.
" 11, 9.40 am. Clear.
" 12, 9 am Perfectly clear.
" 13, 5 P.M. Clear.
" 15, 3 P.M. clear
" 18 noon, Clear.
" 20, noon Clear.
" 21, 4 P.M. Clear Took out and placed at room temp.
" 23, 5 P.M. clear.

April 25. Feebly clouded. Organism unable to grow at temperature of the ice box!

No 9. Ps. stew. (N.Y.) St. 382 (B. fr. Oto phil.). Inoc fr. 13, March 31, 1-3 mm. loop. Thermostat.
April 6, No trace of clouding.
" 7, No cl.
" 8, Clear.
" 9, Clear
" 11, clear.
" 12, Clear
" 13, Clear
" 15, Clear. Removed to room temperature.
" 20, No growth
" 21, Clear.
" 25, Clear.
May 3, 3 P.M. No growth. Organism destroyed by heat of the thermostat.

Fig. 98.*

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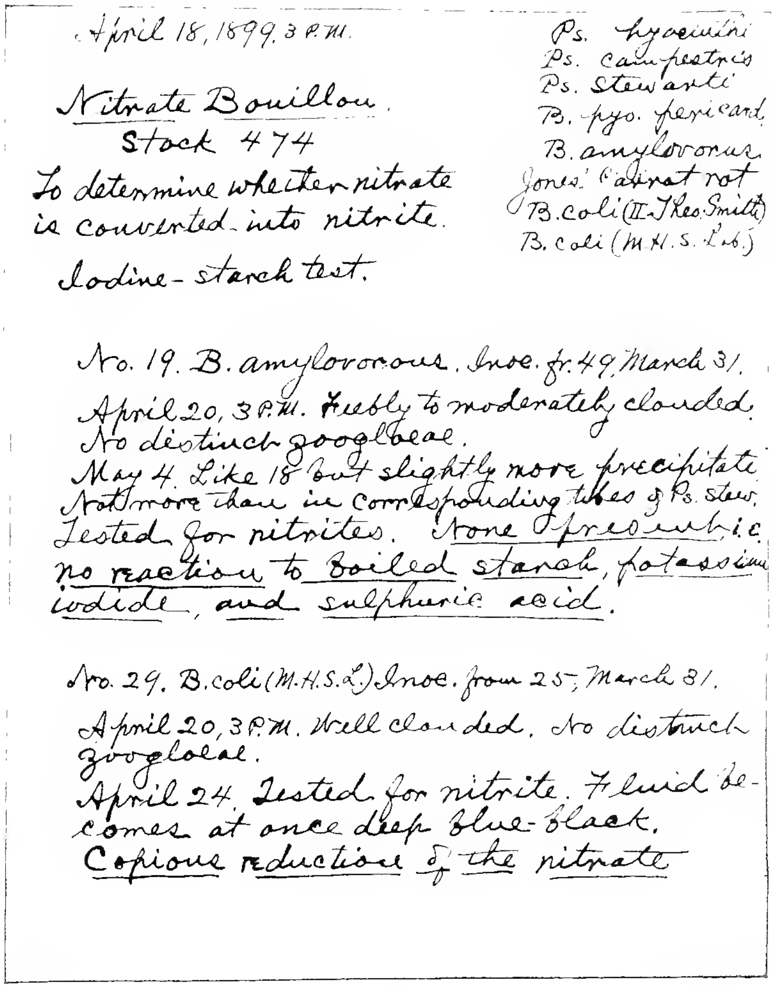
*FIG. 98.—Three sheets showing method of keeping maximum and minimum temperature records. One-half actual size.

After trying various methods, the writer has settled down (in the absence of a stenographer) to the following style of pen and ink notes on cultures, inoculated plants, etc., as extremely flexible and convenient. Reams of ordinary typewriter paper are cut crosswise into three equal portions, so as to form slips about 8 by 3½ inches. As many of these as are necessary for the particular purpose are fastened together at one corner with B, J, N, C, or Z eyelets and the Triumph punch, sold by The W. Schollhorn Company, New Haven, Conn., or by the neat little saw-toothed clamp made by The Middleton P. F. Co., Philadelphia. The first page of the slips is devoted to the name of the organism under examination, the kind of experiment, the date of its beginning, etc. The subsequent sheets are numbered serially and are devoted to particular plants or to particular cultures. If there is an overflow in any particular part of the record, it is very easy to insert additional



April 18, 1899. 3 P.M.

Nitrate Bouillon.
Stock 474
To determine whether nitrate
is converted into nitrite.
Iodine-starch test.

Ps. hyacinthi
Ps. campestris
Ps. Stewarti
B. pyo. pericard.
B. amylovorus.
Jones' Carrot rot
B. coli (II-Theo. Smith)
B. coli (M.H.S. Lab.)

No. 19. B. amylovorous. Inoc. fr. 49, March 31.
April 20, 3 P.M. Feebly to moderately clouded.
No distinct zoogloeae.
May 4. Like 18 but slightly more precipitate.
Not more than in corresponding tubes of Ps. stew.
Tested for nitrites. None of free iod. i.e.
no reaction to boiled starch, potassium
iodide, and sulphuric acid.

No. 29. B. coli (M.H.S.L.) Inoc. from 25, March 31.
April 20, 3 P.M. Well clouded. No distinct
zoogloeae.
April 24. Tested for nitrite. Fluid becomes at once deep blue-black.
Copious reduction of the nitrate

Fig. 99.*

toothed clamp made by The Middleton P. F. Co., Philadelphia. The first page of the slips is devoted to the name of the organism under examination, the kind of experiment, the date of its beginning, etc. The subsequent sheets are numbered serially and are devoted to particular plants or to particular cultures. If there is an overflow in any particular part of the record, it is very easy to insert additional slips. The following transcripts from actual records will serve to illustrate the method (figs. 98 and 99). As fast as the notes are completed they are filed away in boxes or large envelopes until the whole subject has been worked over, when they are sorted out according to their various sub-heads, and all the data which they contain is thus easily available.

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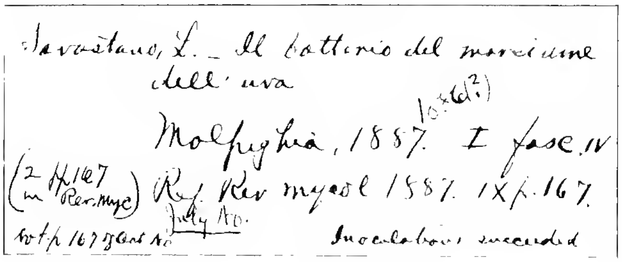
*Fig. 99.—Sheets showing method of keeping nitrate-bouillon records. One-half actual size.

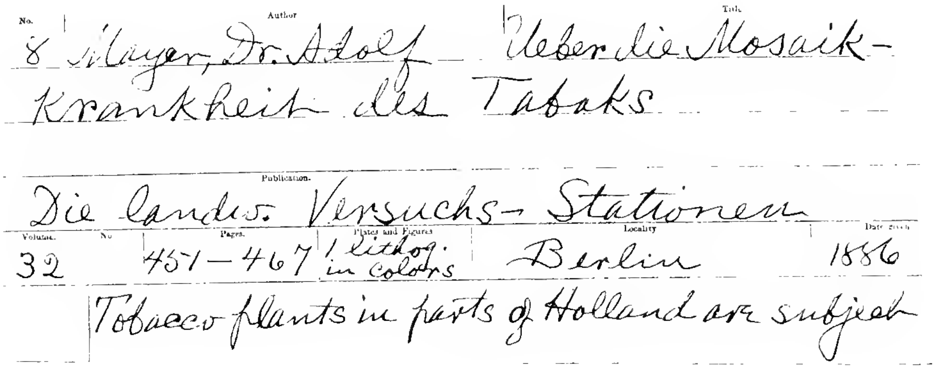
The writer also uses a stenographer whenever possible, and the typewritten sheets, after immediate careful scrutiny for errors of fact, are filed away in stout Manila envelopes with the name of the parasite written on one corner; 16 by 12 inches is a good size for the envelopes.



Savastano, L. _ Il batterio del marciume dell' uva
10×6(?)
Malpighia, 1887. I fasc. IV
(2 H 167 in Rev. Myc) Ref. Rev. mycol 1887. IX p. 167.
July No.
[illegible] p. 167 [illegible] No. Inoculations succeeded

Fig. 100.*

Card-catalogues should be made on the L. B. index slips, made and sold by the Library Bureau, Boston, Mass. Figure 100 is a sample from the writer's catalogue by authors. A larger size should be selected if it is desired to include abstracts. When long abstracts or considerable extracts are made from literature which has been borrowed, or may not be readily accessible in future, heavy sheets (6⅞ by 8⅜ inches) have been used by the writer. These have headlines, as shown in fig. 101, and are preserved by tying into covers made for the purpose. A red line down the left side of the sheet preserves a space for a marginal index.



No. 8
Author Mayer, Dr. Adolf
Title Ueber die Mosaik-Krankheit des Tabaks
Publication Die Landw. Versuchs-Stationen
Volume 32
No.
Pages 451–467
Plates and Figures 1 lithog. in colors
Locality Berlin
Date given 1886
Tobacco plants in parts of Holland are subject

Fig. 101.†

A serious objection to the making of many abstracts is the time involved and the danger of degenerating into a mere student of literature in the effort to make a complete catalogue; another is the fact that, if made in advance of actual need, or by some one not entirely familiar with the subject, it not infrequently happens that the statements in the paper which have been omitted from the abstract as unimportant prove in the end to be the essential ones so far as the owner of the abstract is concerned. For this reason, when they are within reach, the writer prefers to consult the original papers and to save for original work the time consumed in making long abstracts. When they are rare, frequently needed, and only to be had by borrowing, the writer has sometimes photographed the more essential parts. In one instance a pamphlet was borrowed from Europe for this purpose.

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*Fig. 100.—Sample from card-catalogue. Two-thirds actual size.

†Fig. 101.—Top of large sheet used for voluminous abstracts. A red line near left-hand margin marks off a space on which summarizing catch-words or phrases are written. Breadth of sheet, 6⅞ inches.

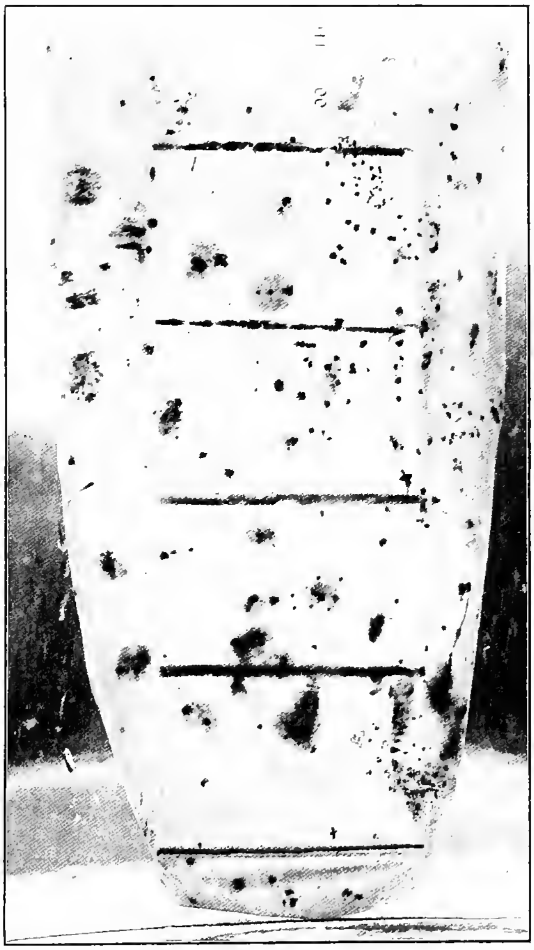

Fig. 102.*

For the exact measurement of colonies, etc., a strip of plate glass 35 cm. long and ruled into 350 mm. spaces may be had from Carl Zeiss, and will be found very convenient (fig. 102).

Steel rules of any size and of very excellent workmanship, graduated according to the English or the metric system in any degree of fineness, may be had from the L. S. Starrett Company, Athol, Mass. Two of these rules much used by the writer are, respectively, 12 inches and 30 centimeters long. They are one inch wide and about three sixty-fourths of an inch thick. They are graduated on both sides, the metric rule into centimeters, millimeters, and one-half millimeters, and the English into inches, halves, quarters, eighths, sixteenths, thirty-seconds, and sixty-fourths.

Stage micrometers made by Zeiss are recommended for the finer measurements. These have 1 millimeter divided into tenths, twentieths, and one-hundredths very accurately. All the magnifications of microscopic objects figured in this book are recorded in terms of such a micrometer. After the drawing has been made it is customary to substitute for the section-slide this stage micrometer and throw the image of some portion of the ruled scale on the paper

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*FIG. 102.—Green cucumber soft-rotted by *Bacillus aroideae*. Contents emptied out and skin filled with water and so photographed, 3 days from date of inoculation, which was by means of a few needle-pricks. The fruit was kept at about 25° C. The black bands are pencil marks on the millimeter rule placed inside. The numerous small dark spots are denser bits of tissue which did not wash free on rinsing out the sack with water. At the left drops of water may be seen oozing through the skin and falling. Photograph, nearly natural size, by Townsend.

where it is drawn, taking care, of course, in case of high magnification, to start one cross line from the outside and the other from the inside of the image of the lines. This method of recording magnifications is urged on all. It takes but a moment, does away with troublesome computations, and enables anyone at any time to determine just what was the magnification. The magnification is determined, of course, by dividing the apparent size by the actual portion of the scale shown. For example, if the scale drawn on the paper is 10 mm. long and represents 0.01 mm. of the actual micrometer scale, then the magnification is × 1000; if it represents the entire millimeter of the micrometer scale, the magnification is × 10.

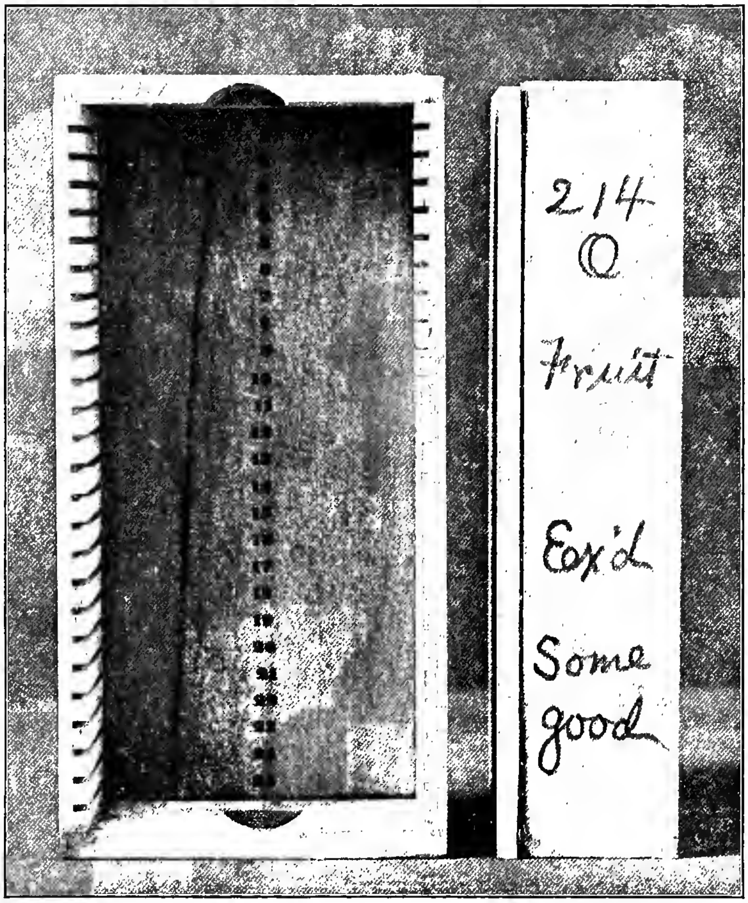

Fig. 103.[*]

For fine weighings, Christian Becker's balances are very satisfactory.

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[*] Fig. 103.—Pillsbury slide-boxes empty and full, made by Bausch & Lomb, Rochester, N. Y. These boxes are simple, inexpensive, and satisfactory, especially for serial sections.

THE MAKING OF COLLECTIONS.

A good, representative collection of diseased material is a prime necessity in every pathological laboratory. This grows into completeness only with the lapse of much time and the aid of many hands. It should include photographs, drawings, paintings, dried material, representative specimens preserved in strong alcohol, and serial sections properly stained and mounted in Canada balsam or Dammar balsam, which must not be dissolved in chloroform, since this gradually removes the stain. With the accumulation of much material, some sort of classification becomes imperative. At present the writer keeps the material designed for sections in 95 per cent alcohol, arranged in as many groups as there are parasites involved. Each jar of material finally receives the same number as the paraffin block from which sections are cut. This material must be examined at least once a year to see that the alcohol has not evaporated, especially if corks are used. Only the best velvet corks should be purchased, and as an additional precaution they should be sealed in with paraffin. The negatives are filed away in similar groups, protected by negative bags. The stained sections, mounted in balsam, are filed away in cheap wooden boxes (Pillsbury boxes), each holding 25 slides (figs. 103, 104). These are very convenient, if properly made, but some boxes of this sort lead to much vexation of spirit, the grooves being too narrow to receive any but the thinnest slides. Those sold in recent years by Bausch & Lomb have given no trouble. In the form shown in fig. 104 the cover remains on better and the mounted slides are easier to take out, but in drying the preparations with the cover off, these boxes tip over at the least touch. During this drying, which requires from a few days to several weeks, the slides should, of course, lie flat, not on edge.

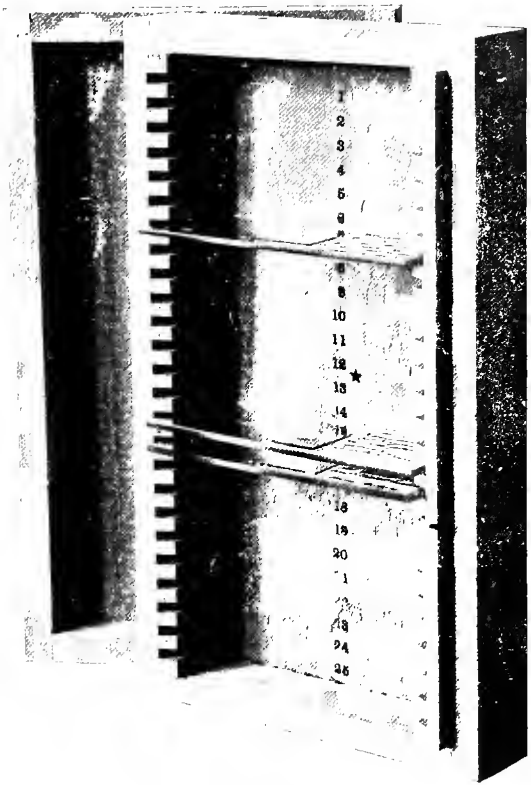

Fig. 104.*

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*Fig. 104.—Another style of slide-box. The advantages of this box are that the cover is not likely to fall off and that the slides, in case of full boxes, are withdrawn more easily. The disadvantages are that it is tipped over very easily when standing on end open, that the cover is readily mistaken for the bottom when it is closed, and that if the cover is put on upside down the writing on the edges is divided. These may also be had from Bausch & Lomb.

The writer passes material designed for sections from alcohol through chloroform (or xylol) into paraffin. Chloroform is preferred in case the infiltration is to be completed in vacuo; otherwise xylol is generally employed. A mixture of xylol and alcohol is first used, then pure xylol, after this xylol with as much paraffin as can be dissolved in it cold. The vial is then placed on top of the paraffin bath and shaved paraffin added until it will dissolve no more at this temperature; the material is then placed inside the apparatus in pure melted paraffin, and it is finally mounted from a second dish of pure paraffin. The temperature of the paraffin bath is usually

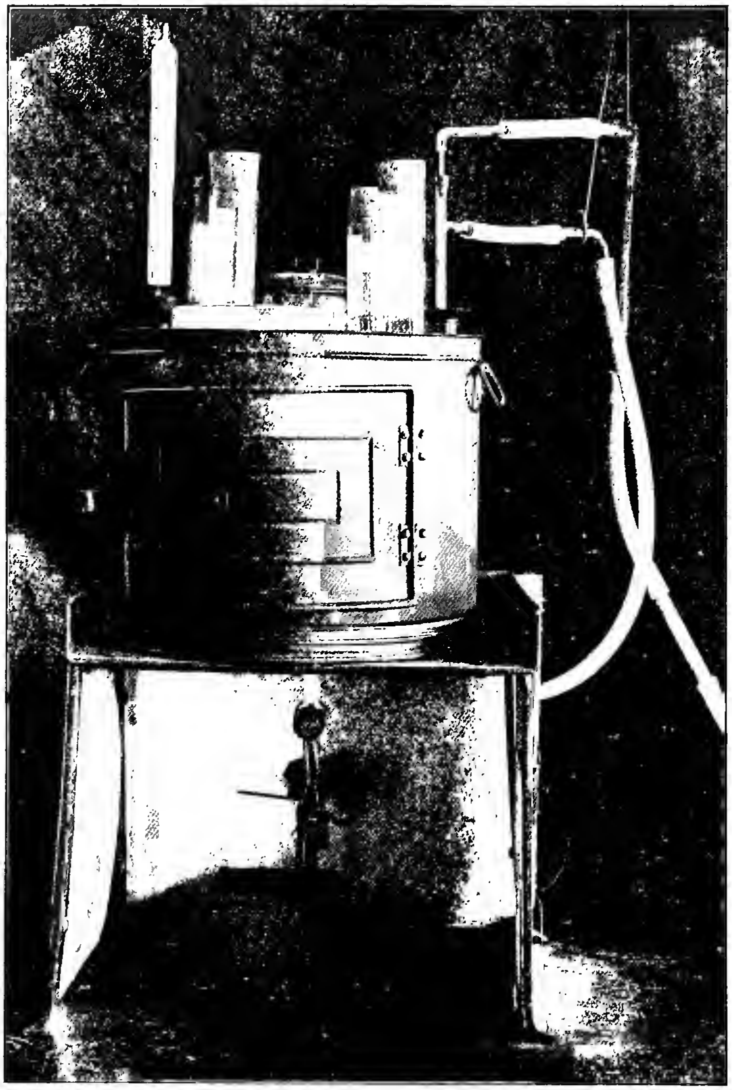

Fig. 105.*

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*Fig. 105 – A small paraffin oven much used in the writer's laboratory. The capacity of the chamber is 6 by 7 by 5 inches. The thermo-regulator is like that shown in fig. 35, but with chloroform substituted for glycerin.

kept at 59° C., and the material is subjected to this temperature only long enough to secure proper infiltration. Generally a few hours are sufficient. A small oven used for this purpose is shown in fig. 105. For large laboratories or classes of students the separate-compartment paraffin oven designed by Dr. Lillie is very convenient. Grübler's paraffin is preferred, and for the climate of Washington we use mixtures of three grades of hardness, viz, melting point 52° C., 58° C., and 60° C., increasing or decreasing the amount of the harder sorts according to the time of year. Dirty paraffin should never be used. All the stock paraffin should be carefully protected from dust. The same remark applies still more pertinently to the sections cut on the microtome. They should be made in still air, in a clean room, and should be carefully protected from dust until stained and mounted. The paraffin-infiltration is usually a simple process unless the material contains air. The embedded material is given a serial number which is scratched on the paraffin (fig. 106), until it is fastened to the cutting block, when it is written on the latter (fig. 107). These blocks are kept as shown in fig. 108. The sections are fastened to clean slides by a *very thin* layer of Mayer's egg albumen fixative (see Lee's Vade Mecum, 5th ed., p. 143), or with pure water, or preferably with 0.5 per cent gelatin water (which will not keep untreated, but may be preserved by adding 3 per cent phenol); the paraffin is removed (after cautious melting) by exposure to turpentine or xylol, alcohol is then substituted, and thereafter graded mixtures of alcohol and water down to alcohol containing 50 or 60 per cent of water, followed by the stain. Water is then removed by passing through graded alcohols into absolute alcohol; xylol or bergamot oil is substituted for the alcohol, and the section is finally mounted in balsam. Coplin's staining jar is preferred (figs. 109, 110). A series of staining jars, ready for use, is shown in fig. 111. The section properly fastened to the slide, and dry, is started in at the left after melting the paraffin with gentle heat, and is taken out at the right ready for mounting in balsam.[‡] In this series of jars the gradations are as follows, beginning

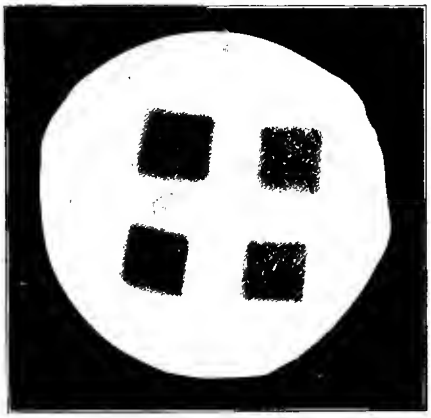

Fig. 106.[*]

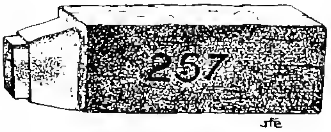

Fig. 107.[†]

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[*] Fig. 106.—Infiltrated tissues embedded in paraffin in a watch-glass and now ready to cut out and mount on blocks for the machine.

[†] Fig. 107.—Infiltrated material embedded in paraffin and mounted on a pine block ready to cut on the microtome. Actual size.

[‡] Sections designed for photo-micrographic work must not only be cut in clean air, but mounted in *absolutely clean balsam*. So much trouble has been experienced in finding such dissolved balsam on the market that the writer now makes his own. The dry balsam is heated in an oven until all easily volatile products are driven off and it becomes brittle. It is then dissolved in xylol and filtered under a bell jar to exclude dust. The filtering usually requires several days.

at the left: Xylol, second xylol, xylol one-third absolute alcohol two-thirds, 95 per cent alcohol, 75 per cent alcohol, 55 per cent alcohol, 40 per cent alcohol, carbol-fuchsin, 40 per cent alcohol, second 40 per cent alcohol, 55 per cent alcohol, 65 per cent alcohol, 75 per cent alcohol, 95 per cent alcohol, absolute alcohol, second absolute alcohol, xylol, second xylol. From this last jar the material is mounted in balsam. Turpentine may be substituted for xylol in jars 1 and 2. After the paraffin is fully removed, the slides are passed rapidly from jar to jar (a minute or two in each being generally sufficient) until the stain is reached. After remaining in the stain the proper length of time (usually three to ten minutes, but sometimes much longer) the slides usually are allowed to remain in the 40 per cent alcohols for a number of minutes, with frequent inspection. When they appear to be properly bleached (rather pale) they are passed rapidly through the remaining jars until they reach the xylol, in which they may remain for some time without injury, if they can not be mounted immediately, but they must not be allowed to stand for any great length of time in any of the alcohols. The secret of success lies in obtaining just the proper amount of differentiation in the 40 per cent alcohol and in not losing any of this later on. To retain the stain it is necessary sometimes to omit some of the graded alcohols.

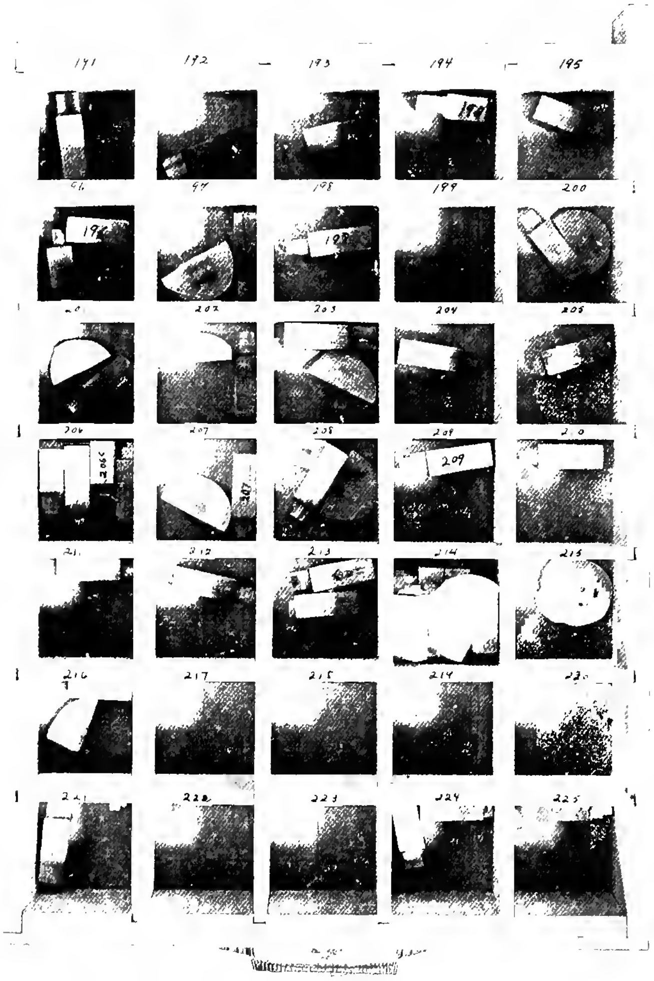
Fig. 108.*

The time required for properly staining sections varies from one or two minutes to a half day or more, according to the subject and the stain employed. No general rule applicable to all cases can be given. When the material is selected for embedding, its serial number, with a full description, is entered in the record book (fig. 112).

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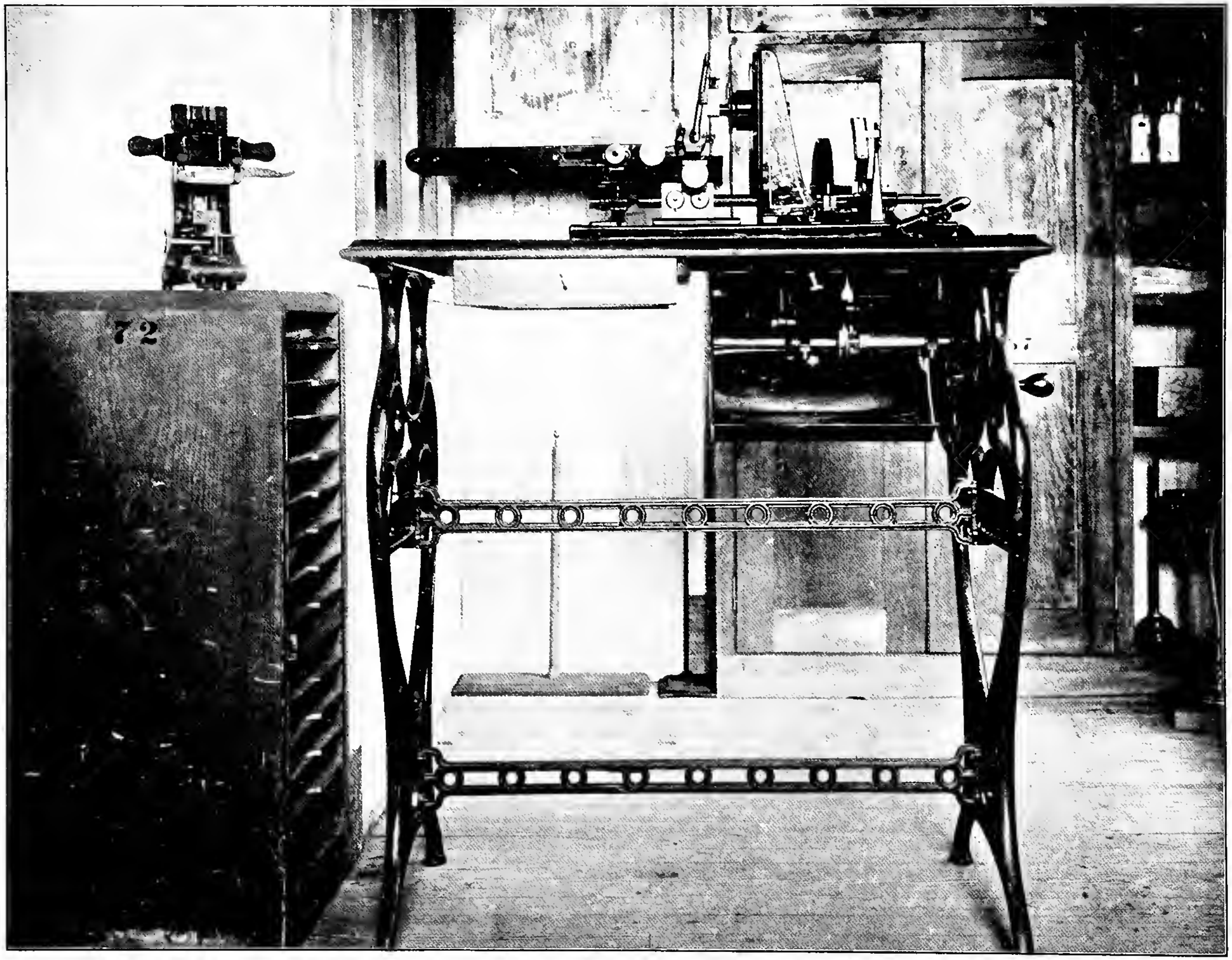
*FIG. 108.—One of a series of drawers divided into small compartments for holding infiltrated, embedded material, cut and uncut.

PLATE 13.



Front view of the Reinhold-Giltay microtome.

Arranged for cutting ribbon-sections from paraffin.

This book must not be lost or misplaced. The advantage of having the serial number written also on the bottle containing the stock of preserved material is very evident if a thing of this sort ever happens. The serial number is written on one edge of the slide-box, and serves to identify it (fig. 103). Some record besides a mere number should also be placed on the slide-boxes. All the slides within bear this number, *e. g.*, 256, and also a series number of their own, *i. e.*, 1 to 25. The slide-boxes are then filed away on shelves either serially or in groups, according to the parasite. Slides containing particularly good fields are marked X, and when the best fields are finally decided upon their location is recorded as determined on the mechanical stage. In case a dozen or more serial sections are included on one slide the the extra good ones are marked X on the first examination, and the others O, as shown in fig. 113. When one of these sections has been drawn or photographed, the X is underscored or inclosed by a circle. This method enables one to keep track of any number of sections. Free-hand sections may be made with the Torrey razor shown in fig. 114D. This is altogether the best razor the writer has used. When very dull it may be sharpened on an India oil-stone. These stones are said to be made of a mixture of carborundum and clay, baked at a high temperature. They may be had of the Norton Emery Wheel Company, Worcester, Mass., in three grades of fineness, the finest being usually coarse enough for the dullest razors. The size needed is 8 by 2 by 1 inch. The finishing may be done on an Arkansas oil-stone, with a

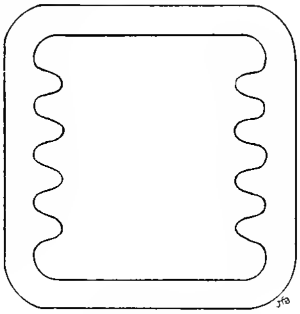


Fig. 110.†

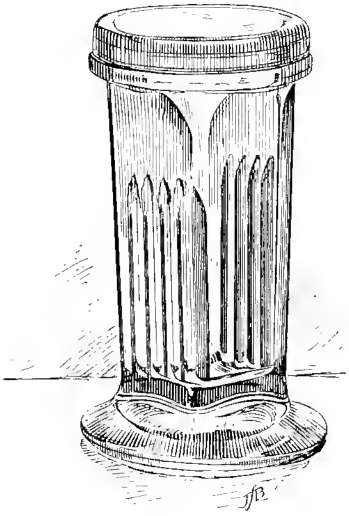


Fig. 109.*

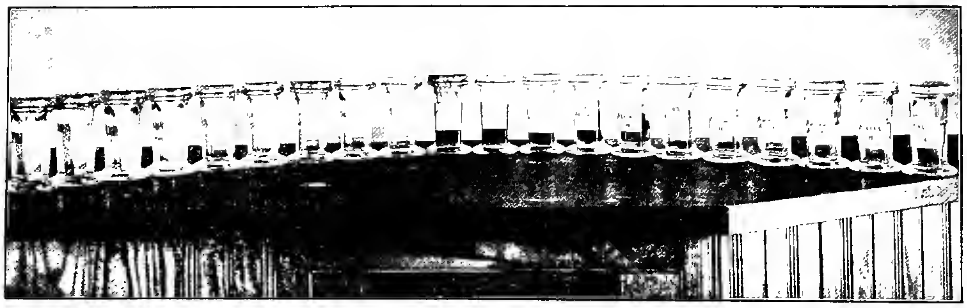

Fig, 111.‡

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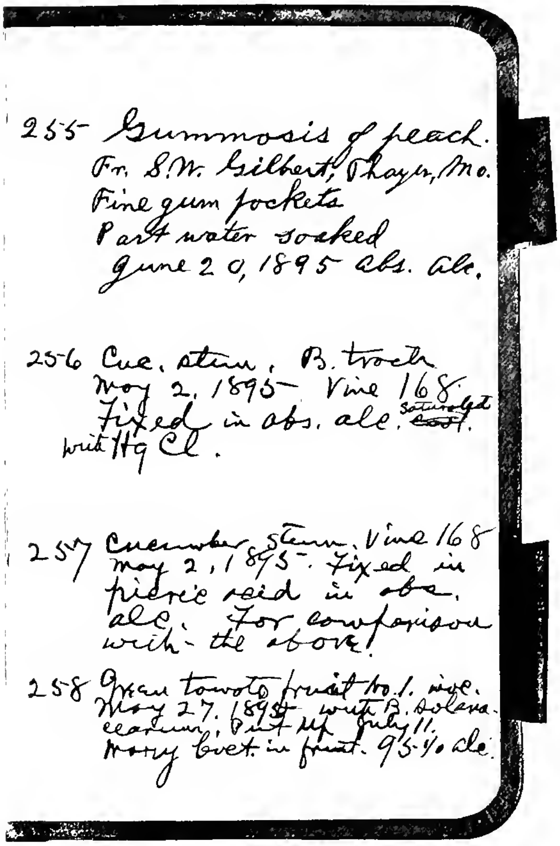
*Fig. 109.—Coplin's staining jar. About one-half actual size.

†Fig. 110.—Cross-section of Coplin's staining jar. About actual size.

‡Fig. 111.—A series of Coplin's jars filled and properly arranged for staining sections fastened to slides.

few final touches on a good leather strop. The maintenance of good edges on microtome knives is a matter of great importance and considerable difficulty, and where much material is to be cut it is very economical of time to send away such knives to be put in order by some expert. In recent years the writer has sent all such knives to Charles Lentz & Sons, Philadelphia, with very satisfactory results. Knives suitable for serial sections are shown in fig. 114 A and C. In fig. 114 B is shown one of a set of knives not inclined to spring and well adapted to the cutting of hard material with a long slant stroke. These knives were made to order by Lentz & Sons at a cost of about $6 each. An end-on view of all these knives is shown in fig. 114 *a*, *b*, *c*, *d*..



255 Gummosis of peach.
Fr. S.W. Gilbert, Thayer, Mo.
Fine gum pockets
Part water soaked
June 20, 1895 abs. alc.

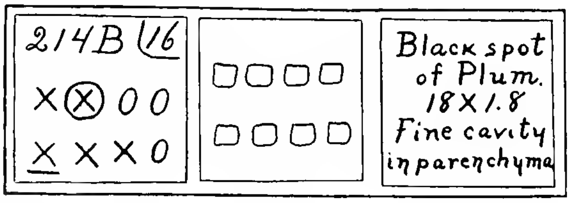
256 Cuc. stem, B. trach
May 2, 1895 Vine 168.
Fixed in abs. alc. saturated ~~sat.~~
with HgCl.

257 Cucumber stem. Vine 168
May 2, 1895. Fixed in
picric acid in abs.
alc. For comparison
with the above!

258 Green tomato fruit No. 1. inoc.
May 27, 1895 with B. solana-
cearum. Put up July 11.
Many bact. in fruit. 95% alc.

Fig. 112.*

Many plant tissues, especially mature leaves, are full of very hard calcium oxalate crystals, and the difficulties of properly cutting such material are very great. The cutting of thin sections of bone would be quite as easy. After even a few sections the edge of the knife looks like a miniature saw and the sections themselves are badly torn, partly by the dulled knife and partly by the movement of the crystals themselves. In case of the yellow disease of the hyacinth the writer has never been able to make satisfactory thin sections, many of the soft cells being filled with bundles of very hard raphides which he has not been able to dissolve without serious injury to the tissues. In such cases thick free-hand sections are about all that can be hoped for.



214B 1/6
X ⊗ 0 0
X X X 0

Black spot
of Plum.
18 X 1.8
Fine cavity
in parenchyma

Fig. 113.†

Serial sections are cut on the microtome. The one shown in pl. 13 and fig. 119 leaves nothing to be desired in the way of a perfect-working durable instrument. The ribbon-carrier is above the table at the left. The knife is stationary. The block moves up and down, and the razor-carrier moves forward at each stroke a distance governed by the set-screw of the scale (½ μ to 40 μ). By substituting a wide knife-carrier, sections several centimeters in diameter may be cut, and by using a slanting knife, as for celloidin, very hard material may be cut. By loosening a set-screw, the razor as here shown may be turned a few degrees to right or left, and the paraffin block may also be moved through a considerable arc in any direction, it being held securely in any position by pressure of a collar-screw on a ball-and-socket joint. On 72 in plate 13 is an apparatus for trueing the edges of the paraffin blocks.

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*Fig. 112.—A page from the paraffin record-book. The numbers on the slide-boxes (fig. 103) correspond to numbers in this book. Two-thirds actual size.

†Fig. 113.—A mounted slide of serial sections, showing manner of labeling.

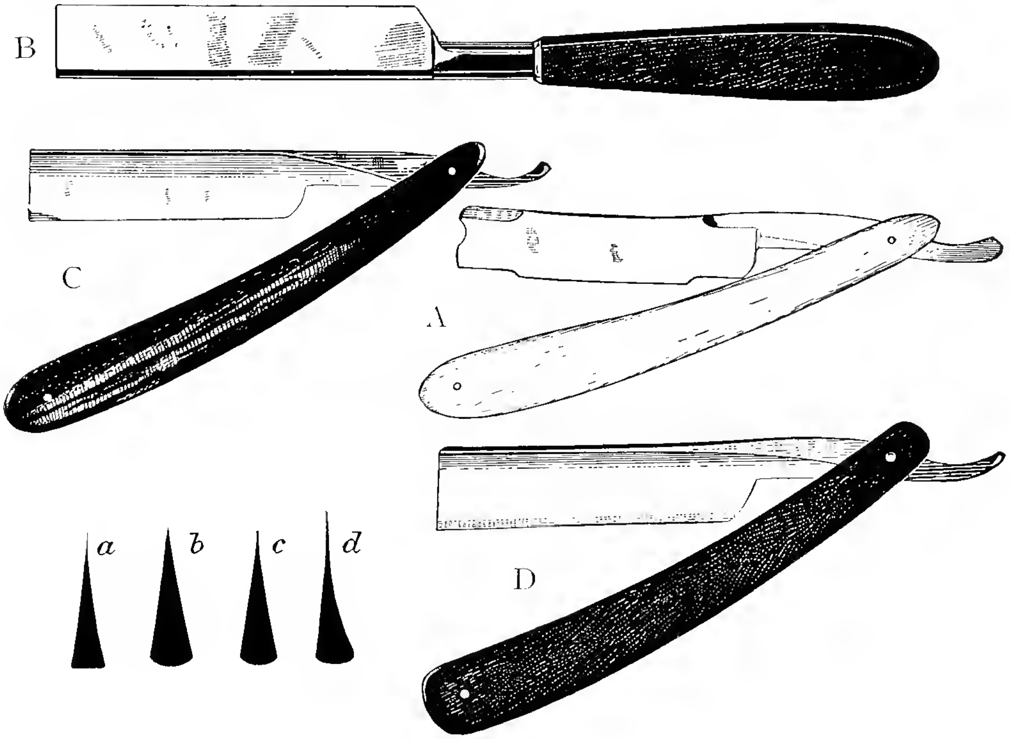

Fig. 114.[*]

Collections of living bacteria are also necessary. Fortunately many may now be obtained, as needed, from Král, in Prague; but, unfortunately, they do not always correspond to their name. Others must be kept on hand, and the cultures (of some sorts) must be renewed at frequent intervals. That way which has given the writer least inconvenience is by storage in cool boxes (refrigerators) at temperatures of 10° to 15° C. By this method some organisms can be kept alive on agar a year without transfer, and even sensitive organisms will generally live for some months, especially if planted in proper media. The writer has never made any attempt to prepare a collection of dead bacteria on culture media to serve as museum specimens, but it is possible to do so, it is said, with considerable success by following the methods described by Hauser and others (Bibliog., LII).

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[*] Fig. 114.—A. Knife for serial sections, furnished with the Reinhold-Giltay microtome. This is made by Joseph Rodgers & Son, Sheffield, England. One-half actual size.

B. Microtome knife made to order by Charles Lentz & Sons, Philadelphia, and found useful in cutting hard material with long slant strokes. One-half actual size. The broad wedge-shaped blade of this knife is shown in *b*.

C. Knife obtained from J. R. Torrey & Co., Worcester, Mass., and found very useful for making serial sections on the microtome. One-half actual size.

D. Torrey razor, recommended for free-hand sections. The very thin blade is flat on one face and hollow-ground on the other, as shown in *d*. It is made of the very best steel and holds an edge well. One-half actual size.

a, b, c, d, end views of the cutting edge of knives shown in A, B, C, D. Actual size.

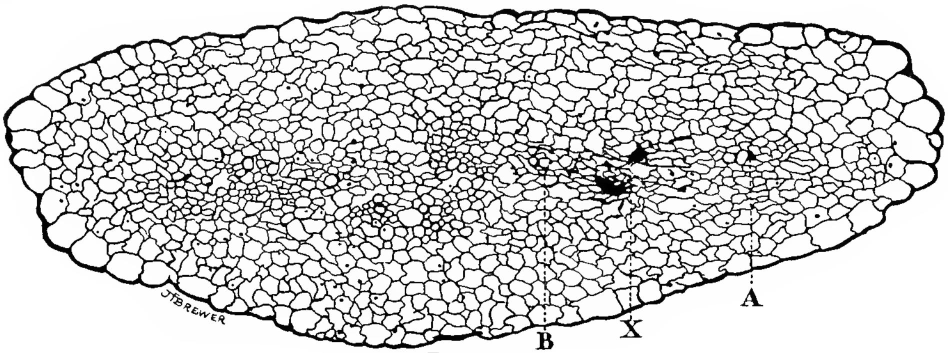

Fig. 115.*

DISTILLED WATER.

All laboratories doing much work should have an abundance of distilled water, and where this is not readily obtainable in sufficient quantity and of good quality, provision should be made for it when the laboratory is constructed or when the necessity for it arises. In the construction of such a still many things must be kept in mind, if it is to work satisfactorily and yield water of the desired purity.‡

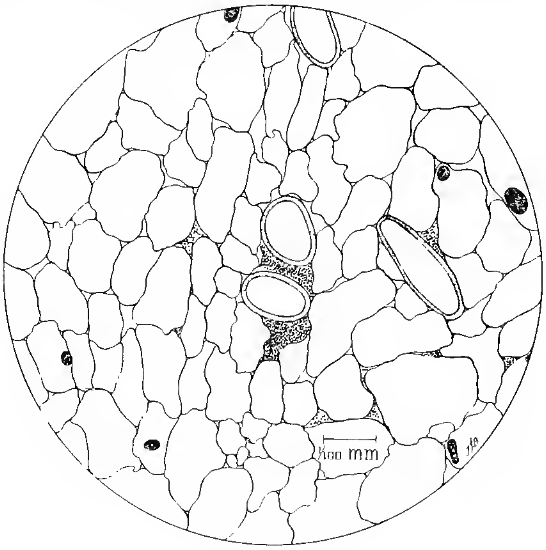

Fig. 116.†

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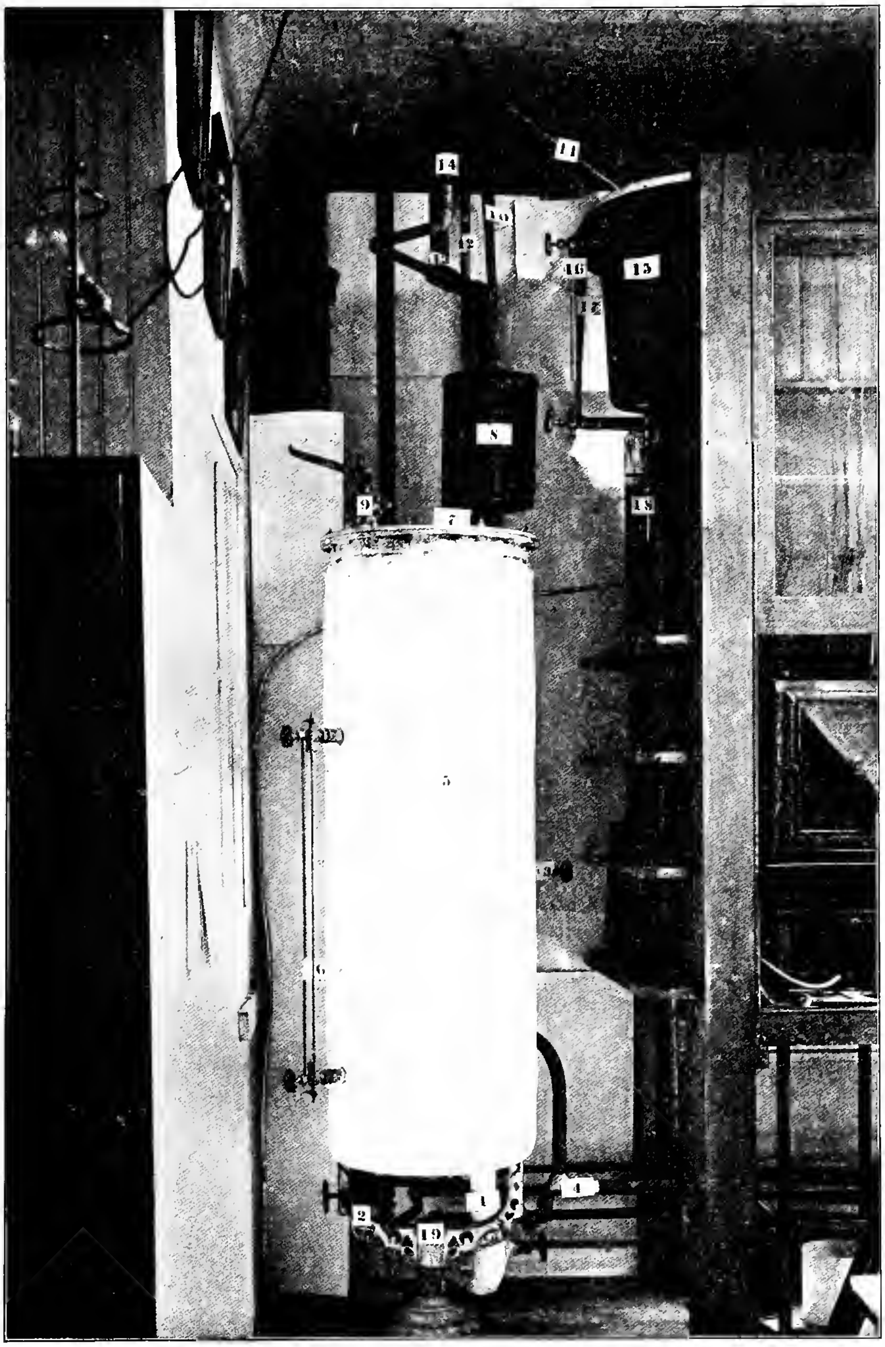
*Fig. 115.—Cross-section of tooth of cabbage-leaf infected by *Bacterium campestre*. Plant No. 401 sprayed with water containing an agar-culture. Bacterial occupation limited to points between A and B. At X vessels are occupied. At A and B the bacteria lie in the intercellular spaces and have not yet entered the vessels. For details of A and B, see figs. 116 and 117. This section, which is one of a series, was cut 270 μ below the apex of the leaf-tooth. A few micromillimeters further down (370 μ) all trace of the bacteria disappears. In other words, the bacteria are still confined to the leaf-tooth, and there is no cavity like that shown in fig. 76. When sprayed this leaf was extruding fluid from the water-pores. Actual length of section, slightly under 1 millimeter. Slide 331c 3 Plant sprayed December 9, 1904; slightly blackened leaf-tooth fixed in 95 per cent alcohol on December 17, 1904. Inked from a photomicrograph.

†Fig. 116.—Cross-section of leaf-tooth of cabbage infected by *Bacterium campestre*. A detail from fig. 115 at A. The bacteria have not yet entered the vessels.

‡That thing which has given the writer most trouble was an entirely unexpected difficulty, viz, a plague of tiny red house ants. These got into the reservoir in spite of all that could be done to render it tight, and, of course, spoiled the water for all delicate work.

PLATE 14.



Apparatus for Distilling Water.

(1) Steam inflow pipe; (2) waste-steam pipe; (3) hydrant-water inflow pipe; (4) hydrant-water outflow pipe (flush) to sewer; (5) galvanized-iron boiler; (6) water gage; (7) brass top, tinned on the under side; (8) copper catch basin; (9) steam safety valve; (10) block-tin steam pipe to condenser; (11) block-tin water pipe from condenser; (12) hydrant-water pipe into condenser tank; (13) hydrant-water pipe from condenser tank; (14) flush pipe for condenser tank; (15) reservoir, capacity 80 gallons; (16) water gage; (17) overflow pipe from reservoir; (18) block-tin pipe leading to various rooms; (19) iron support.

The following description and figure of a distilled-water apparatus devised by the author for use in the Laboratory of Plant Pathology, United States Department of Agriculture, may be of interest, therefore, to some. The apparatus consists of a galvanized-iron boiler similar to those used in kitchen ranges. It is 18 inches in diameter and about 5 feet high. The top is sawed off and to it is bolted a stout iron ring with a flange, on which rests a ¼-inch brass cover. In the lower half of this boiler is a coil of 52 feet of inch copper pipe, the upper end bent downward and *securely fastened* in the bottom of the boiler to a steam pipe (1 inch) connected with a 1½-inch steam pipe leading to the ordinary steam boiler in the engine room in the basement; the lower end connected with an iron steam pipe (1 inch) leading to a steam trap (Mark traps are said to be the best). Around this copper steam pipe, which is of course tin-plated, stands the river water which is to be converted into steam by contact with the hot pipe. This hydrant water is kept always at about the same level (level of fig. 5 in plate 14), by means of a tinned-copper ball float (automatic cut-off) which closes the mouth of the inflow pipe when the water rises beyond a certain point. The upper part of the cylinder is a steam chamber under very moderate pressure (0 to ½ pound, rarely more). The excess of pressure is dissipated either by escape of steam through the safety valve (9), which is not weighted, or through the coil of pipe in the condenser. The steam passes from a securely riveted tin-lined copper catch basin (8) into a ¾-inch block-tin pipe (10), which is fastened to a tubular projection from the catch basin by means of a collar screw. The tubular projection from the top of the catch basin is soldered in place and also held by a flange inside the copper top, so that it can not be forced out by any attainable degree of steam pressure. The ¾-inch block-tin pipe passes to the room above, where it is coiled for a length of 35 feet inside a tin-lined copper tank resting on the floor. The height of the condensing tank is 18 inches and its diameter is the same. When in operation this tank is full of running water. Theoretically, this condensation tank is large enough, and it is so practically when the hydrant pressure

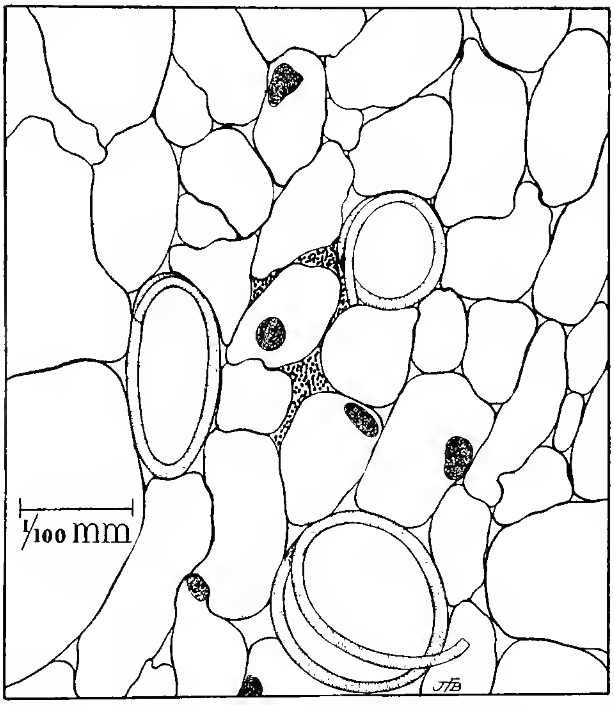


Fig. 117.*

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*Fig. 117.—Detail from fig. 115 at B, showing an early stage of water-pore infection of cabbage. The bacteria have not yet entered the spiral vessels. The large dark bodies are nuclei.

is good, but when it is feeble or when the steam pressure is high the water becomes too hot and steam sometimes escapes into the reservoir. The water therefore must be hurried through the tank by the use of a steam pump, or else less steam must be allowed to enter the copper pipe. If the writer were to build another similar apparatus he would make the condensing tank 2 feet higher and add 10 feet to the length of the coil of tin pipe. The condensing tank is provided at the bottom with a 1-inch inflow pipe for the cold water (it should be 1½-inch), and at the top with a 1½-inch outflow pipe (it should be 2-inch), for the exit of the warmed water. There is also a 1-inch flush pipe at the bottom for the occasional removal of sediment.

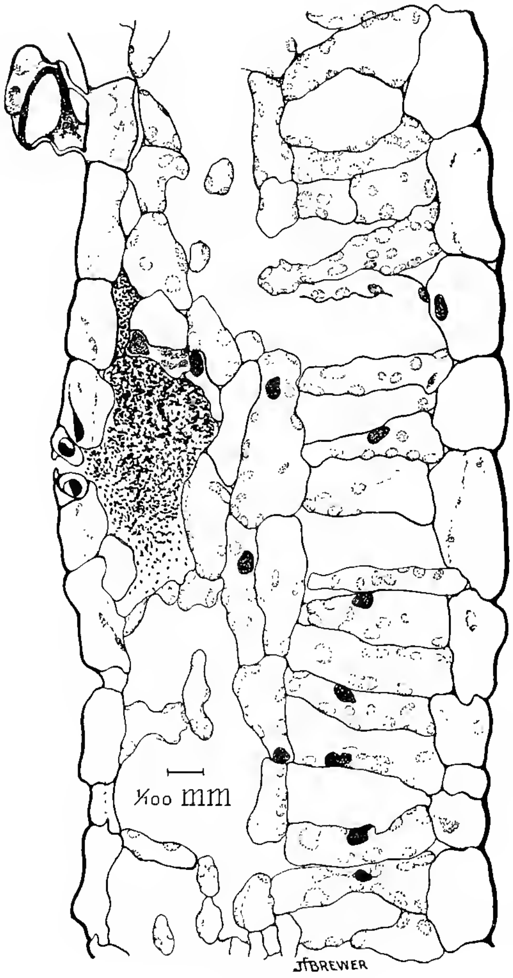

Fig. 118.*

The size of the outflow pipe, which must be somewhat larger than the inflow pipe, prevents any possibility of clogging and overflow. All the metal parts which come into contact with the distilled water are tinned or nickel-plated. Connected with the lower end of the block-tin coil (by tin solder, which must not contain lead or zinc) is a smaller (½-inch) block-tin pipe (11), which leads the distilled water into (15) the storage tank (¾-inch pipe would be better, and without any joint). The reservoir in this case is a white-enameled bath-tub, on the top of which is clamped down a cover of thin sheet copper ($\frac{1}{60}$-inch), the inner face of which has been carefully tinned. Plate glass ground to fit would be better, and the tub itself is likely to be discarded in the near future, *i. e.*, when some more satisfactory storage tank can be found. The problem of the proper storage of distilled water in quantity is the hardest one, the solvent power of the water is so great. From the bottom of this bath-tub several hundred feet of ½-inch block-tin piping lead to various rooms in the building. In addition to the terminal faucets there is a general cut-off just above 18, which is necessary in case of an accident to any faucet or part of the piping. There is also an overflow pipe (17), which does not enter the sewer, but

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*Fig. 118.—Early stage of stomatal infection in angular leaf-spot of Rivers cotton. Hothouse infection produced by spraying *Bacterium malvacearum* upon the surface of the leaves. For a much later stage see fig. 80.

ends free in the laboratory about 1 foot above a deep sink. The sides and top of the boiler, the copper catch basin, and the $\frac{3}{4}$-inch block-tin pipe leading to the condenser are all coated with 3 inches of best non-conducting magnesia covering. The catch basin, designed to hold back solid particles carried up with the steam, is 9 by 12 inches and is made of $\frac{1}{16}$-inch copper, securely riveted and soldered with tin solder. It is bolted down to the flat brass top and a steam-tight connection is secured by means of a red rubber gasket. The heavy brass top (7) is tinned on the inner surface and is bolted securely to the iron flange on the top of the boiler by means of 18 screw-bolts. The junction is made steam-tight by means of a corrugated

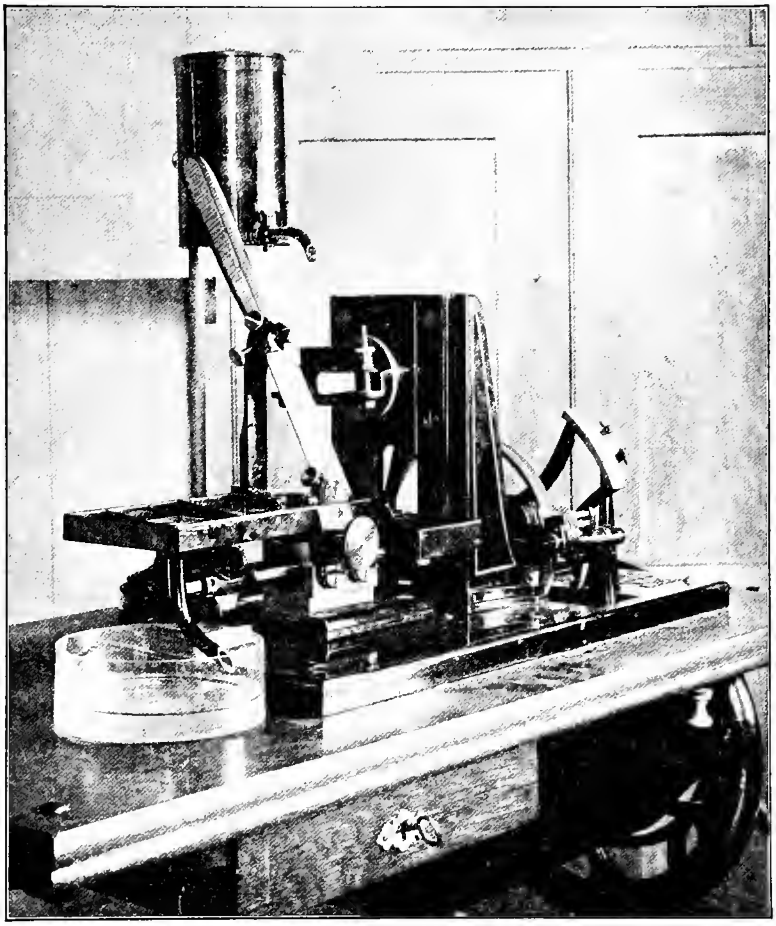

Fig. 119.*

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*FIG. 119.—The Reinhold-Giltay microtome arranged for cutting celloidin or very hard paraffin sections. The machine is very solidly and accurately constructed out of the best materials, and, in addition, provision is made by means of set-screws for compensating the wear due to long use. The device governing the thickness of the sections is especially ingenious. This particular machine has been in constant use by various persons for over four years, and nothing has been paid out for repairs. With good use it ought to last a lifetime. About one-fifth actual size.

tinned-copper gasket. The steam which runs the apparatus is brought to the laboratory floor through a 1½-inch pipe, in which (in the engine room) there is a steam gage registering up to 150 pounds, and a reducing valve set at 55 pounds. This very considerably lessens the steam pressure in the copper coil, moderates the violence of the ebullition, and makes the apparatus perfectly safe. The hydrant-water outflow pipe (flush) to the sewer, for occasionally washing out accumulated mud (4) passes from the bottom of the boiler immediately above fig. 19. Gate-valves are used. All brass and copper parts in contact with the steam are tinned; all metal parts in contact with the distilled water are tin, tinned, or nickel-plated.

With 60 pounds steam pressure in the engine-room boiler, 40 pounds pressure at the reducing valve, 35 pounds pressure in the pipe at the laboratory floor near where it enters the still, and one-half pound pressure or less in the steam chamber above the coil of copper pipe, the capacity of this still is 60 liters (16 gallons) per hour.

The apparatus must be built very substantially in all parts, so as to withstand at least twice as much steam pressure as any part of it will be subjected to, *e.g.*, 160 pounds in the iron pipes and in the copper coil and its attachments, and at least 20 pounds in the catch basin, and other parts subject to steam generated in the still. A steam gage, in addition to the one in the engine-room, shows the pressure in the coils, and another the pressure in the steam chamber above the coils. They are not shown in the plate, as they were put on after that was made. The former is attached to the steam supply pipe near the floor, and the latter to an arm of the safety-valve pipe. The boiler should be taken down and the parts retinned once a a year, or at least once in two years.

If a much greater quantity of water is needed the block-tin condensation coil should be lengthened to 60 feet, the diameter of the inflow pipe of the condenser should be increased to 2 inches, and the outflow pipe to 2½ inches, and the cubic contents of the condenser tank should be quadrupled. The capacity of the bath-tub (or other receptacle), for a large laboratory should be at least 500 liters, and might well be 1,000 liters.

The above apparatus has been in use for two years. It works very smoothly and satisfactorily when the proper amount of steam is let into the coil of copper pipe, which ordinarily should not be nearly the whole amount available. The inflow of steam is governed by the valve a few inches below fig. 1 in plate 14. When too much steam enters the coil, the pressure in the steam chamber above it rises to five pounds or more, hot water is forced back through the feed pipe (3) into the neighboring pipe which furnishes cold water to the condenser (12), and steam instead of distilled water is furnished to the water tank. This is at once obviated by cutting off part of the steam inflow and moderating the force of the boiling. It might also be obviated by reducing the length of the arm of the safety valve (9), which in any event should not be weighted.

Sufficient water for small quantities of culture-media and pure enough for most purposes may be obtained from the simple glass still shown in fig. 82 by one distillation. Water of a high degree of purity may be obtained by two distillations, adding 0.5 gram to 1 gram of potassium permanganate per liter of water before the first distillation, and 5 grams of c. p. sulphuric acid per liter before the second distillation. The flasks in which such water is collected or stored should be of resistant (non-soluble) glass and absolutely clean to begin with. With use such flasks or bottles become more valuable and should not be employed for other purposes.

The solubility of glassware is best tested by determining from time to time the degree of electrical conductivity of pure water stored in it. The specific resistance of pure water stored for a week in such tubes, flasks, or bottles should not fall below 250,000 ohms. The specific electrical resistance is determined upon 1 cubic centimeter of water exposed between electrodes having an area of 1 square centimeter, and is read by means of a special Wheatstone bridge. Distilled water redistilled with chromic-acid cleaning mixture, and afterwards with alkaline potassium permanganate (method used by the Physical Laboratory in the Bureau of Soils) gives a resistance of 700,000 ohms.

The following determinations made by the Physical Laboratory of the Bureau of Soils show the diverse behavior of two lots of clean test-tubes recently purchased as non-soluble glass by the Laboratory of Plant Pathology.

Kind of tube.	Time of exposure, in days.	Specific resistance, in ohms.
Resistant test-tubes,(R) from Greiner & Friedrichs	10	220,000
Do., 2d test	11	219,000
Tubes received from the School Supply Co	10	41,400
Do., 2d test	11	34,000

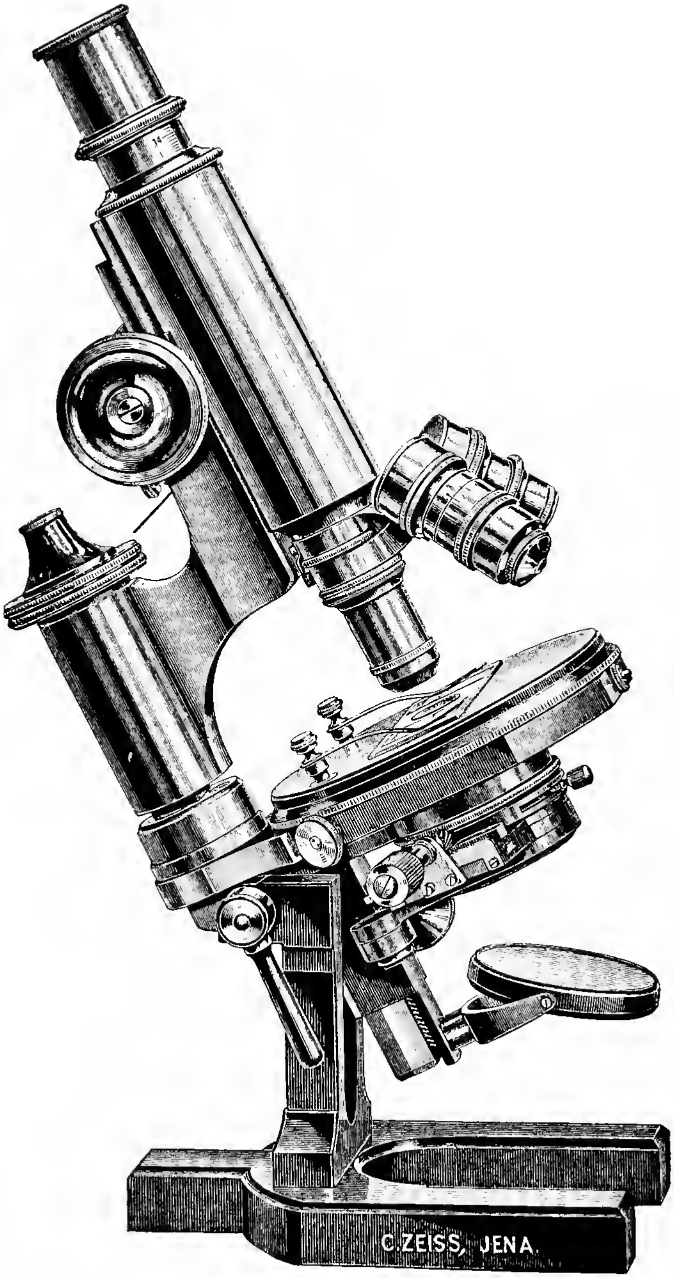
The twice-distilled water used was taken from a Jena flask and its initial specific resistance was 240,000 ohms.

MICROSCOPES.

Microscopes of a much better grade are required for bacteriological investigations than for ordinary histological work. The writer has for many years employed those made by Carl Zeiss, of Jena, as, on the whole, most serviceable. Good microscopes are also made by E. Leitz, of Wetzlar, and recently by the Spencer Lens Company, of Buffalo, N. Y. The Zeiss stand shown in plate 15 does very well for all ordinary work, but is not well adapted for the making of photomicrographs or for recording the exact location of particular spots in the section. The latter difficulty may, however, be overcome by means of a removable slide-carrier attached to the stage. The stand may also be used with the small upright photomicrographic outfit shown in fig. 24 when the lens does not require a microscope having a wide tube. This microscope has a half-mechanical stage, an excellent fine adjustment, and good substage apparatus. It is thoroughly well made and very durable. One in the writer's laboratory has been in use for twelve years. The lacquer has disappeared in places and it is no longer attractive to look at, but it has required no serious repairs during this time and is still serviceable.

For photomicrographic work and also for recording the exact location of desirable fields in a section, the writer uses the large photomicrographic stand shown in plate 16. This is provided with a specially wide barrel, a fine adjustment of very

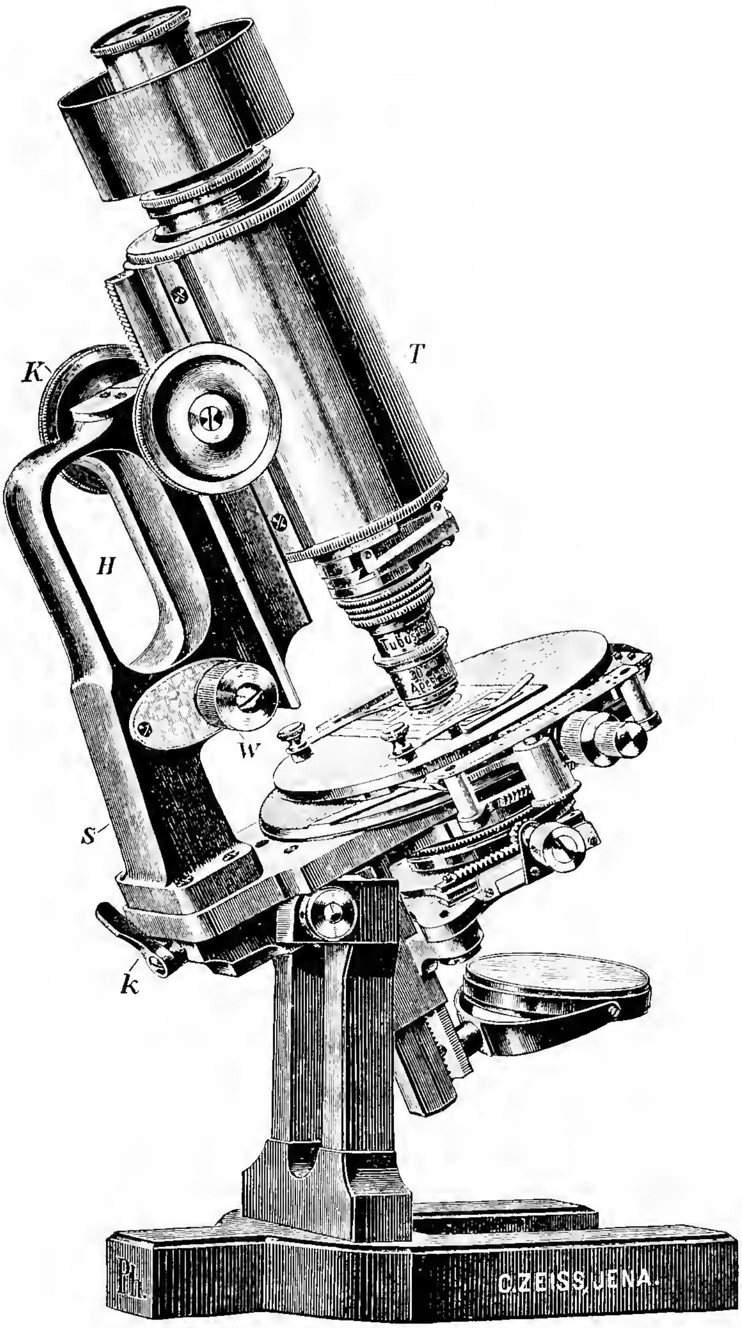
PLATE 15.


C.ZEISS, JENA.

Zeiss microscope stand II^{a}.

This form of microscope and that represented on plate 16 are the two patterns used principally in the Laboratory of Plant Pathology, U. S. Department of Agriculture. The objectives are apochromatic, and have proved very serviceable. In carrying do not grasp by any part above the level of the stage, as this brings an undue weight upon the fine adjustment. Seize by the base.

PLATE 16.



Zeiss photomicrographic stand I^{c}.

The barrel "T" is of greater diameter than in stand IIa. The fine adjustment is at "W" and no weight rests on it in lifting the instrument by the handle "H." The set screw "K" locks the upper part of the instrument at any angle. The objective is set in place by means of a very convenient slide carrier. The fine adjustment screw has an extremely slow movement; and the vernier screws are on the same axis (a great convenience). The stage rotates and may be locked at the desired place by means of a set screw. For the substage arrangement see figure 120.

slow movement, a swing-out condenser (fig. 120), two substage iris diaphragms, and various other conveniences. For example, the screw-heads, determining the cross and sidewise movement of the section, are on the same axis and may be reached and moved without changing the position of one's arm.

The apochromatic objectives are the only ones recommended for bacteriological work. They cost more than achromatic objectives, but expense is a minor consideration. In hot, moist climates the older apochromatic objectives of Zeiss frequently became clouded, but those made in recent years have given the writer no trouble in the latitude of Washington. They yield sharp images even with high eye-pieces. Of course, compensating oculars must be used with the apochromatic objectives. It is desirable to have the whole series of objectives and eye-pieces, but if one is limited for means, very good work can be done with two objectives and three oculars, viz, objectives 16 mm. and 3 mm. 1.40 n. a., and compensating oculars 4, 6, and 12.

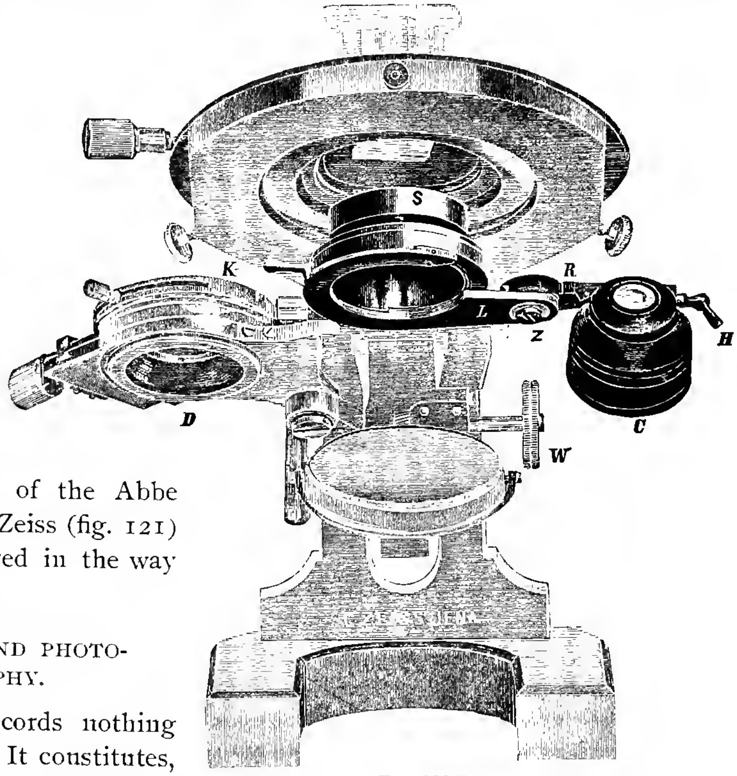


Fig. 120.*

The newer forms of the Abbe camera furnished by Zeiss (fig. 121) leave little to be desired in the way of a drawing camera.

PHOTOGRAPHY AND PHOTOMICROGRAPHY.

For permanent records nothing equals photography. It constitutes, therefore, a very important special part of laboratory work, and every student of pathology should make a knowledge of this subject part of his education. Some of the following suggestions will be useful to beginners.

The Zeiss Double-Protar lenses, series VII*a*, are the best all round photographic lenses made by that firm, and are excelled by none made by any firm. The back or front lens is usually as good as the combination. Excellent photographic lenses are also made by Voigtlaender and by Goerz. Zeiss photographic lenses may be

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*Fig. 120.—Swing-out condenser and other substage arrangements on Zeiss photomicrographic stand, No. 1*c*. There is an iris diaphragm in D, and a second one in S, which is for use when the condenser is thrown out as shown in this figure. D swings under when C is thrown into place. W racks the entire substage up or down.

obtained from Bausch & Lomb, who are under contract to manufacture them according to the Zeiss formulæ. In buying a photographic outfit it is economy to get one of the high-priced lenses. It is frequently stated, by those who do not know, that "just as good results" can be obtained with cheap lenses, but one may easily satisfy himself that such is not the case by photographing buildings on a street or any object having many vertical parallel lines and other lines crossing at right angles. The pictures made by the cheap lenses generally show serious distortions. In buying a lens one should know in advance exactly what he wishes to do with it, otherwise he may be greatly disappointed. If he wishes to photograph only

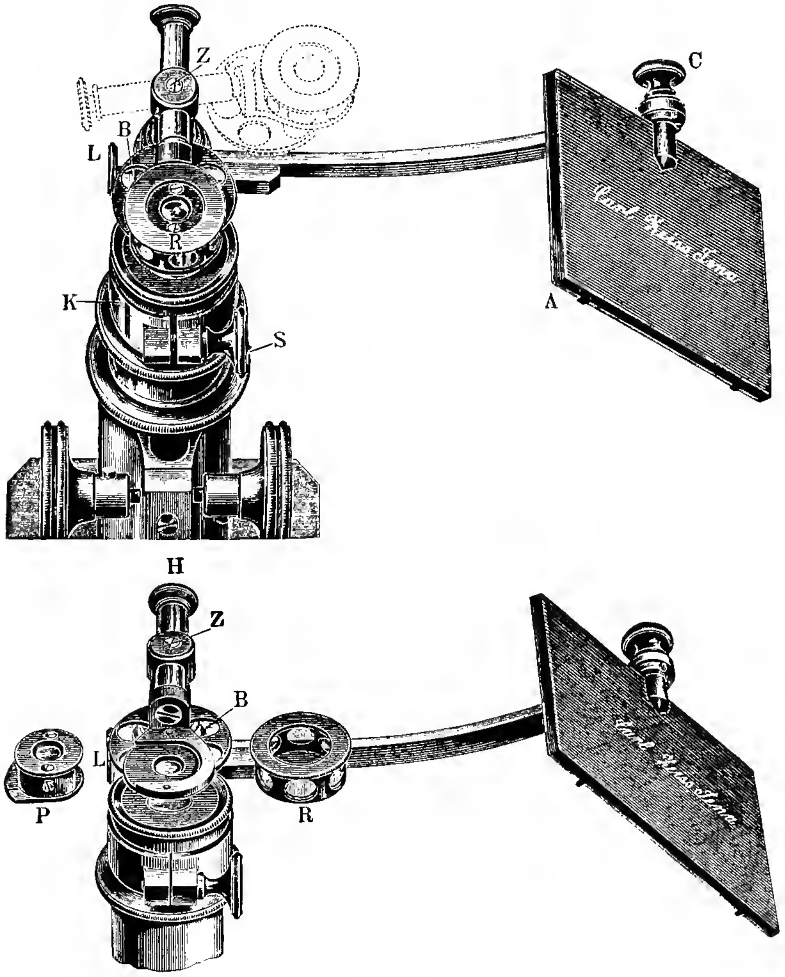

Fig. 121.*

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*Fig. 121.—Newer form of Zeiss-Abbe drawing camera. The camera is clamped at K by means of S. The prism within R is centered over the eye-piece by screw movements of L and Z. When not in use the prism is swung to the right, as indicated by the dotted lines. The mirror A throws down the prismatic image to the drawing paper. The amount of light is governed by the substage iris-diaphragm and by rotating B and R, which contain smoky glasses of graded densities. P is an extra prism. The image on the paper will also be clearer if it is placed in shadow by means of a screen of some sort.

flat surfaces he will select a lens with no great penetration, but with a very clear field, sharp to the edges, *i. e.*, a Planar or some similar lens. If he needs a lens with very little depth of focus (but more than the Planar) and one allowing dark objects to be photographed in a very short time, *e. g.*, luminous bacteria by their own light, he will select a Zeiss Unar or its equivalent, *i. e.*, an extremely rapid lens. If he desires in one picture as much as possible of a landscape, *e. g.*, a large tree or an interior, he will select an extremely wide-angle lens rather than one distinguished for its rapidity or for the perfection of its definition, *e. g.*, a Zeiss Protar, series V. The Double-Protar, series VII*a*, combines as wide an angle, as flat a field, as great rapidity, and as sharp a definition as it is possible, apparently, to obtain in a lens and at the same time have great depth of focus. These lenses may also be unscrewed and each half used separately, if one wishes some portion of a picture more highly magnified. They are furnished with front and back lenses of equal or unequal focal distance, as may be desired.

In using Planars and all lenses which magnify, it is necessary to secure *a very exact focus with the stop wide open*, for, unlike lenses which give pictures less than actual size, only a very little increased depth of focus can be obtained by stopping down. With many objects—*e. g.*, the surface of a leaf, or of bacterial colonies—there is considerable difficulty in deciding which is the proper focus when a Planar is used, what seemed like a good focus often yielding a poor negative. On this account the writer is in the habit of focusing on a fragment of very fine, sharp print laid on the surface of the leaf or of the agar-plate near the colonies to be photographed. A lens magnifying 6 times is used in judging of the image on the ground glass, and when the best possible focus has been secured, the paper is removed, the lens is stopped down two-thirds, and the photograph is made. In case of white colonies the best results are obtained by resting the Petri dish on a piece of black paper while the photograph is being made. The exposure is shortened by illuminating the surface of the object with a bright beam from a mirror. The apparatus

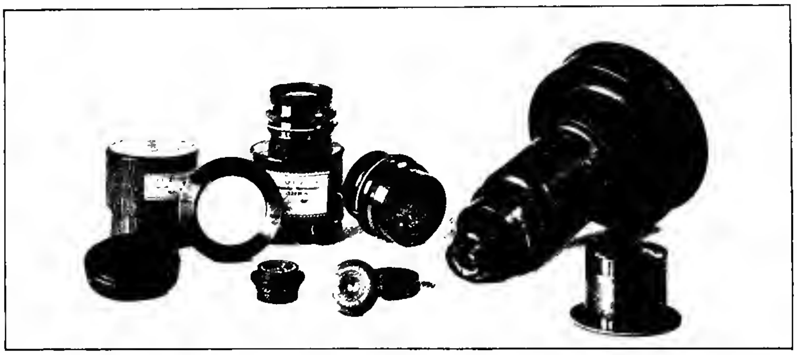

Fig. 122.*

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*Fig. 122.—Zeiss Planar lenses, series I*a*, Nos. 1 to 5. Nos. 1, 2, and 3 may be attached to the funnel-shaped carrier shown in the figure. This screws into the top of the microscope barrel in place of the eye-piece tube. The one attached is No. 3. The condensing lenses necessary for these Planars are also shown in this figure, at right and left.

shown in fig. 24 may be used for this purpose. To avoid shadows the mirror should be held some distance above the object when the surface is not even. The first five of the Zeiss series of Planars are all that are usually required. No. 1 gives the highest

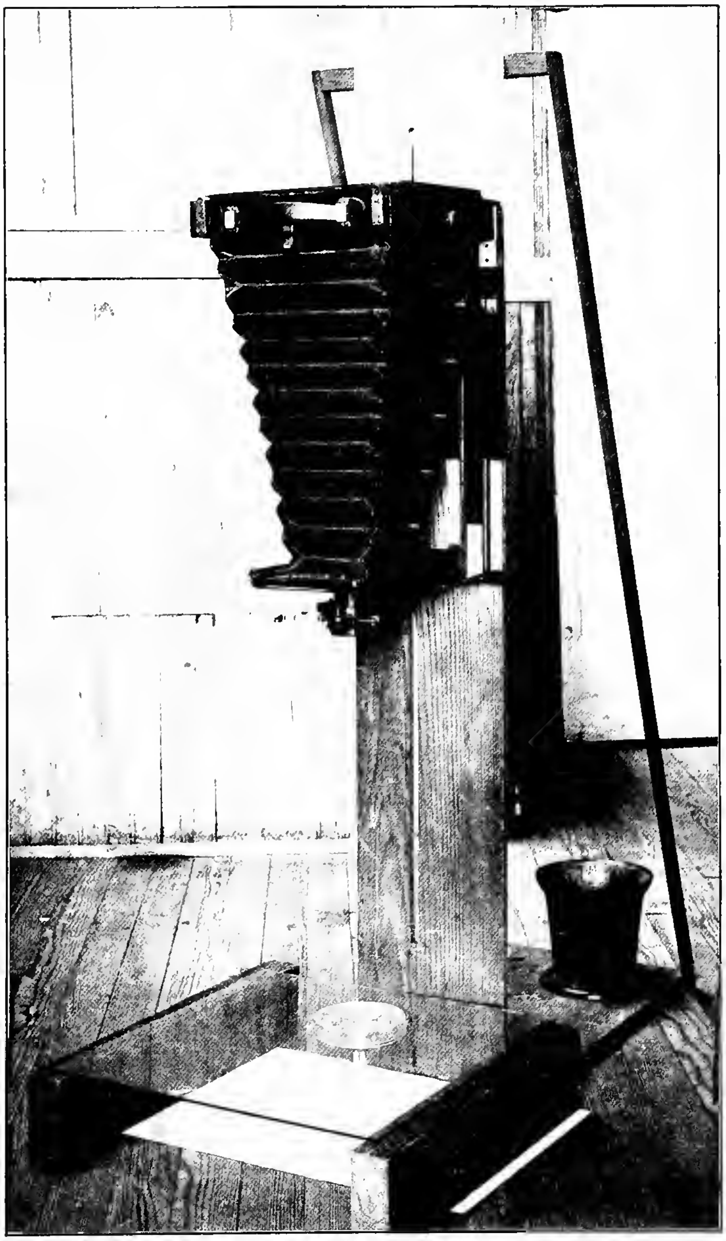

Fig. 123.*

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*Fig. 123.—Simple apparatus for holding the camera in place when one wishes to photograph down. The camera here shown is a Rochester Optical Company, reversible back 5 by 8, fitted with a Bausch & Lomb rapid universal lens, and has been used very often by the writer for natural-size work and for lantern slides.

magnification; No. 5, the largest field; No. 3 will give a sharp image of a flat object a centimeter in diameter. Special condensing lenses are required. These fit into the substage in place of the Abbe condenser. One condenser serves for Nos. 1, 2, and 3, and another for Nos. 4 and 5 (fig. 122).

In photographing poured-plate colonies natural size, there are several ways. It may be done by reflected light, as shown in fig. 123, in which case the colonies sometimes cast deep shadows. Such shadows may be avoided by mounting the camera as shown in fig. 124 and gently twirling it during the exposure. The Petri dish may also be photographed by transmitted light exactly as if it were a negative for a lantern slide. The Petri dish is then held in place in the darkened window or in front of the camera box by crowding it into a hole cut in a square of thick leather, paper, or sheet-rubber ($\frac{1}{8}$ inch), which is then fastened over the kit or framework by eight thumbtacks, or, better, it may be held in place by two stout rubber bands, as shown in the photographs (plate 17 and fig. 125). With stop 32 u. s. and Seed's 27-X plates the right exposure in Washington is usually somewhere between $\frac{1}{5}$ second and $\frac{1}{2}$ second in sunny weather and 3 to 5 seconds in cloudy weather, using a Voigtlaender collinear lens, series III, No. 6, and south light.

Atkinson gets very good results by

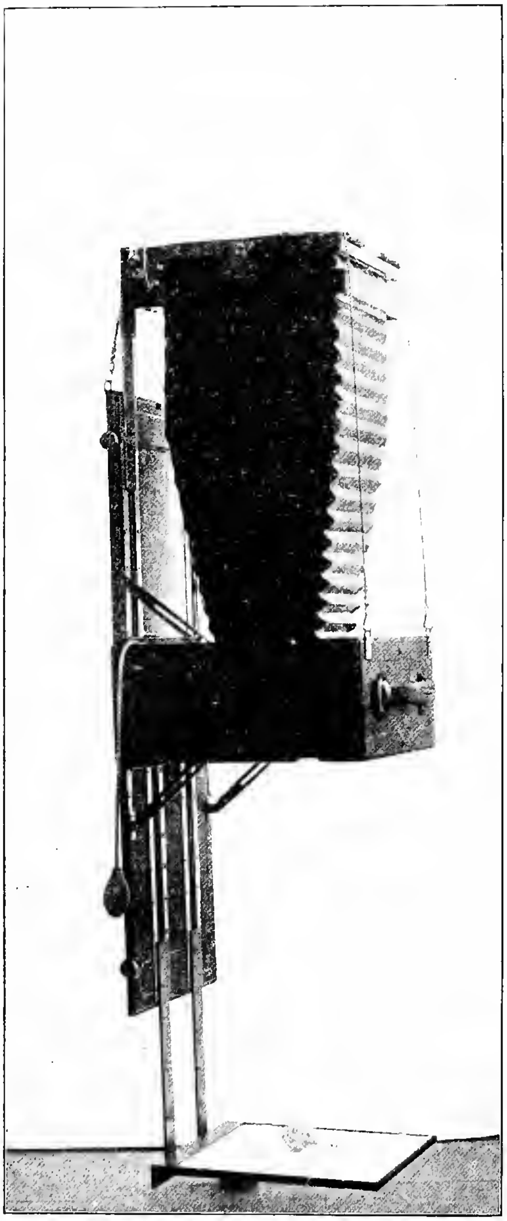
Fig. 124.*

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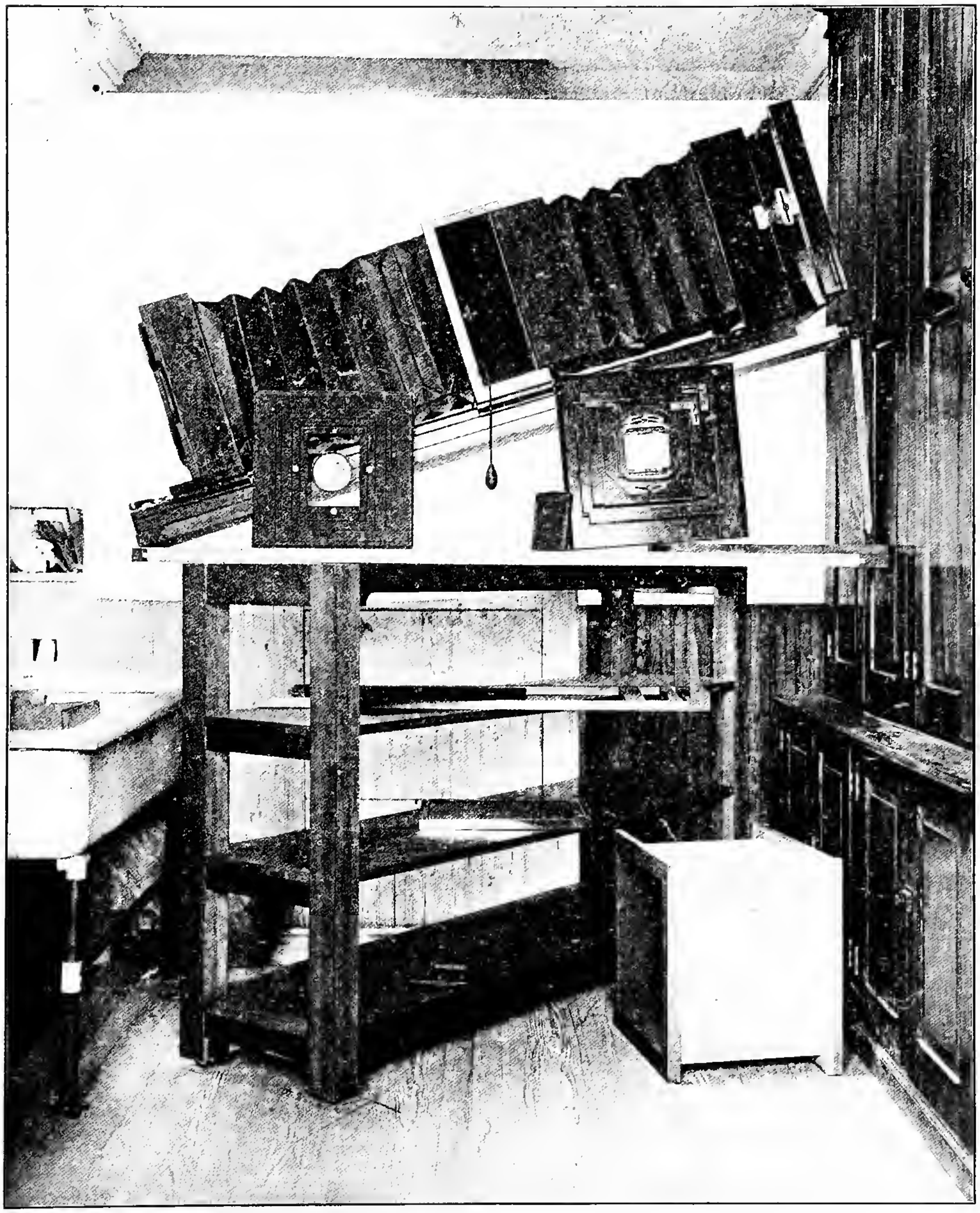
*Fig. 124.—Modified Collins-Brown camera swung from the ceiling and set to magnify about × 1¾. The four suspending strings, which are of very strong fish-line, end in an S-shaped hook, the upper end of which hooks over a ring attached to a stout cord pendant from the ceiling. The length of bellows in this camera as modified by the writer is 25 inches. The lens used with it is a Zeiss Double-Protar, Series VII*a*, No. 13, made by Bausch & Lomb, Rochester, N. Y. This is the type of lens known also as the Zeiss Convertible Double Anastigmatic. This lens has a focal distance of 9¼ inches, or, when only the front or back half is used, 16½ inches (16 according to Zeiss catalogue). It is provided with a Bausch & Lomb No. 2 Volute shutter. A cork support was placed under the object carrier to steady the apparatus while it was being photographed, but in actual use the camera swings free, and if one desires to avoid shadows the apparatus is given a gentle twirl just as the exposure begins. The object carrier is easily removed, and is held in place at any level by two set-screws.



Enlarging and reducing camera, showing method of mounting the apparatus.

On the table at the left is a Petri-dish poured plate held in place by two rubber bands and ready for photographing. On the table at the right is a specia camera-back used in making lantern slides. This allows the ground glass to be raised or lowered, pushed to right or left, or rotated at will.

placing a circular black disc centrally some distance behind the plate to be photographed, using for illumination the diffused light which comes in around this disc. The result is a very sharp contrast, *i. e.*, white colonies on a black background (Bull. Torrey Bot. Club, 1893, Vol. XX, p. 357).

In photographing test-tube cultures the chief trouble is the great number of confusing high-lights due to the curved surface of the glass. From an artistic standpoint these are to be desired, but inasmuch as they are sometimes liable to be mistaken for bacterial growths the naturalist desires to eliminate them. This may be done in several ways. One of the best ways is to photograph the tubes through a thin sheet of distilled water. For this purpose jars of clear white glass are necessary. These should be about $5\frac{1}{5}$ wide × $5\frac{3}{4}$ deep × $1\frac{3}{16}$ inches thick (inside measure), with parallel walls and a flat bottom. Such jars may be obtained of Emil Greiner. Only those without flaws or wavy lines should be accepted. Better jars with perfectly parallel flint-glass walls may be had from Carl Zeiss. Good results

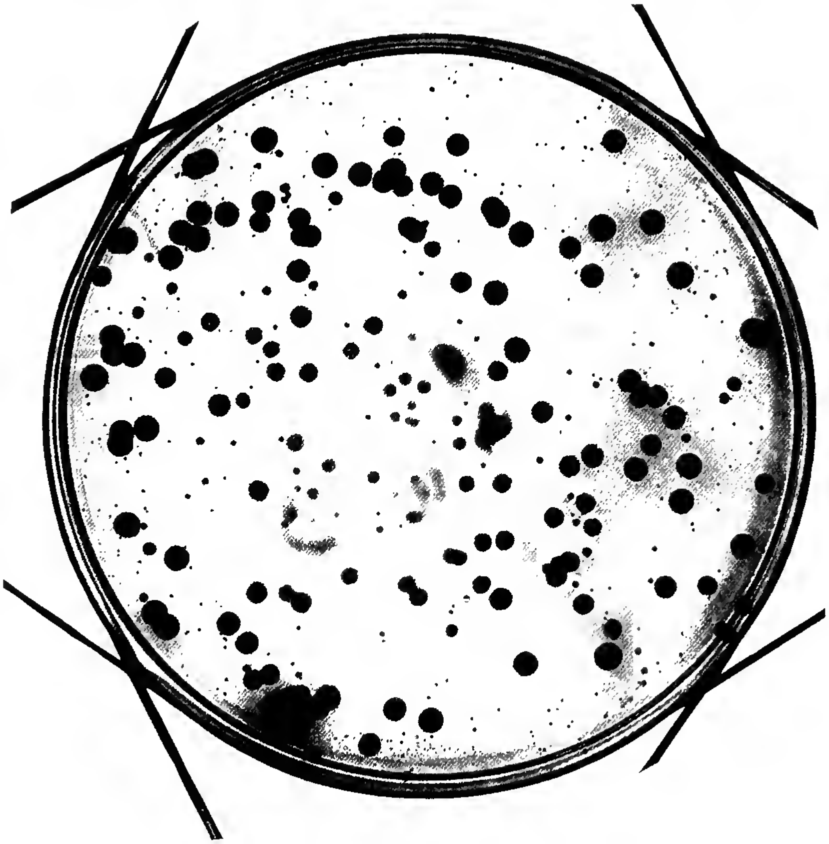


Fig. 125.*

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*Fig. 125.—Showing method of holding Petri-dish poured plates for photographing by transmitted light with camera shown in plate 17. The dish is held in place by two stretched rubber bands, exactly as if it were a negative to be used for making lantern slides. For manner of suspension, *i. e.*, relation to the camera, see plate 17. The organism is a 48-hour agar culture of van Hall's *Ps. syringae* II, grown at 24° to 27° C.

may also be obtained by photographing the tubes against a north light inside a box with blackened walls. The box may be 8 × 8 × 12 inches, open at each end, and painted inside with a mixture of lamp-black and turpentine. One open end is pointed to the window, the other to the lens of the camera. In the middle of the box, crosswise, on top, a row of ¾-inch holes (1½ inches apart) is bored, and the tubes to be photographed are thrust through these. If images of outside objects appear on the ground glass, they may be cut out by pasting white tissue-paper on the window-glass (this should be glued only at the corners).

The kind of plate used depends upon the object. If the contrasts are very great—*e. g.*, a waterfall or bright rock surrounded by vegetation, or an interior with the camera pointed toward windows—a double-coated non-halation plate should be used (Hammer's Aurora plates are very good; plate 6 was made with such a plate). In the absence of such plates most of the halation may be avoided by squeegeeing to the back of the dry plate, before loading, a black paper soaked in glycerin (plate 14 was made in this way). The dry plate should be placed face down on dry blotting paper during this process, and, of course, the glycerin-soaked paper must be cut in advance to fit the dry plate. Much may be done during development to avoid violent contrast if one knows how, the quantity of pyrogallol or ortol being greatly reduced and the development prolonged. This gives a thin negative full of detail.

For many purposes isochromatic plates are invaluable; for other purposes ordinary plates will give better results. Which kind is best adapted to a particular subject will depend on what is wanted. In general, for this sort of work, the full contrast of the original is desired, and the kind of plate which will give it is best. Even some exaggeration of the contrasts of the original is not objectionable in many cases if the prints are to be used for half-tone reproductions, since contrast is often reduced in the half-tone process, and there must be exaggeration in the photograph if the half-tone picture is to correctly represent the object. An example or two will help the beginner to judge. If we have black spots on a green background and use a rapid non-isochromatic plate, we will get the result shown in fig. 126, which may be contrasted with fig. 127, made from the same leaf, but on an isochromatic plate. For such cases the isochromatic plate is of course the one to be selected. In the same way, if one desired to reproduce the variegations of a pansy with the full value of each color, he would use an isochromatic plate. On the contrary, white spots or stripes on a green leaf, or yellow colonies on an agar or gelatin plate, or red spots on a white ground, will stand out better if the photograph is made on a Seed's 27-X non-isochromatic plate or its equivalent. Red spots on a green background require an isochromatic plate. Black spots on a yellow or orange ground usually require for good contrasts an isochromatic plate. Some yellows, however, take pale, while others take dark.

In making photomicrographs little trouble is experienced with low powers, but there is considerable difficulty in making good negatives of bacteria in tissues, using high powers. A few hints may be of service. With upright stands and certain objectives the beginner frequently has difficulty in securing a uniformly lighted field. This trouble may be obviated by throwing the light from the mirror not directly on the substage mirror of the microscope, but on a sheet of ground glass (it may be the focusing plate of the camera) placed in front of the mirror of the microscope. The coarse adjustment of the microscope should not work too easily, or else the mere weight of the microscope tube may throw out the focus after it has been secured and before the picture can be taken. The connection between camera and microscope must be light-tight. In absence of a proper device (foot of stand in fig. 24), light may be cut out by several folds of black velvet pinned close. The stage of the microscope should also be protected from bright reflected light when photographing by transmitted light. If there is a rigid connection between the camera and the top of the microscope, or if the latter rests on the base of the former, the focus is apt to be injured by slight jars incident to putting in the plate-holder or drawing the slide. For this reason it is better to have them separate, and the carrier and draw-slide should be scraped, sandpapered or filed, and waxed, soaped, or vaselined, so as to work very smoothly. An entire day spent in accomplishing this end should not be counted as wasted time.

With large horizontal cameras (plate 5) the work-table and the bellows-table must be leveled up accurately with reference to each other, sidewise as well as vertically, and then must be bolted to the floor. The order of apparatus beginning at the window is: mirror, condensing lens, alum-cell, light-filter (Zettnow's fluid),* microscope, automatic shutter, front board of the camera, large black diaphragm in middle part of bellows, ground glass of the camera. The newer styles have a screw-device for elevating or lowering the camera and another for elevating or lowering the microscope, or the optical bench. Dr. Novy has added to his Zeiss table a very convenient device by means of which the services of an assistant are dispensed with, one person behind the ground glass of the camera being able from this position to move the slide in any direction desired. The cost of the attachment is about $15.

Beginning with the center of the mirror at the far end of the work-table or beyond it, and ending with the center of the ground glass at the back of the camera, all parts of the apparatus must be *centered* accurately, *i. e.*, the light reflected from the center of the mirror must pass in a straight line through the center of the condensing lens, Abbe condenser, objective, and eye-piece to the center of the ground glass at the back of the camera, otherwise a first-class negative will not be obtained. The Abbe condenser must also be at the right distance from the stage of the microscope; the image will then be on the center of the ground glass, circular, uniformly lighted, and free from distortion and color fringes, if the optical parts are in proper working order. The distance of the Abbe condenser varies, of course, with the objective. The Planar lenses require special substage condensers, such as those shown in fig. 122. When the centering is perfect all the rest is easy, or becomes easy with a little experience. If sunlight is used, an automatic shutter should be placed on the end of the camera next the microscope, so that accurately-timed short exposures may be made. The sun's rays should pass through several inches of fluid before they enter the objective; otherwise, if the focus of the condensing lens should accidentally coincide with the balsam mount of the lenses for a few minutes, it may be softened and the objective ruined. Pure water is as good for this purpose as alum water, which was formerly much recommended. It removes more than 50 per cent of the heat rays.

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*This is a mixture of copper nitrate and chromic acid in distilled water. It lets through only the greenish-yellow rays. This fluid acts on the cement of the flint-glass container, and should not, therefore, be allowed to stand in it longer than necessary. The latter should then be washed in pure water and properly drained.

The writer uses a Zeiss 3-inch mirror with micrometer-screws for throwing the sun's rays. This serves quite as well as the more expensive heliostat, if one can work quickly. The order of procedure is to obtain the proper focus and see that it "holds;" the plate holder is then introduced and *opened*, and consequently the bellows must be light-tight; last of all, the sunlight is accurately re-centered and the shutter snapped. The photomicrograph should be made with light from the central

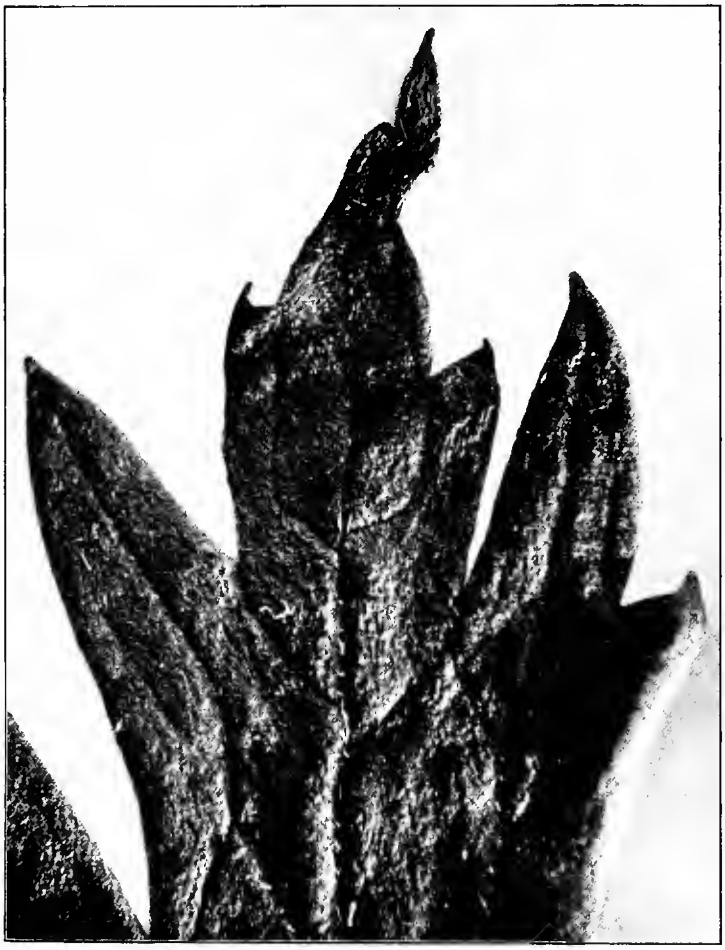

Fig. 126.*

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*Fig. 126.—Fragment of a green leaf bearing black spots. Enlarged 6½ times with a Zeiss Planar lens and photographed on a Seed's 27-X plate. Introduced for comparison with fig. 127. Notice that although stopped down considerably, part of the leaf is out of focus.

portion of a considerable image of light. My custom is to nearly close the iris diaphragm below the Abbe condenser and throw with the condensing lens a small circle of light into the center of this diaphragm; the condensing lens is then slid along the track about 12 or 15 inches nearer; the iris diaphragm is then opened wide and the exposure made at once by squeezing the bulb of the shutter.

I now always use apochromatic lenses and never make negatives without an eye-piece. I have used Zeiss projection oculars, but now use in preference a Zeiss No. 4 compensating ocular, or Spencer No. 3, which is kept solely for this purpose (so as to be always clean). It is of the utmost importance that mirror, walls of light-filter, alum-cell, and surfaces of condenser, slide, objective, and ocular be absolutely free from dirt, grease, and dust particles, even the smallest, if a good negative

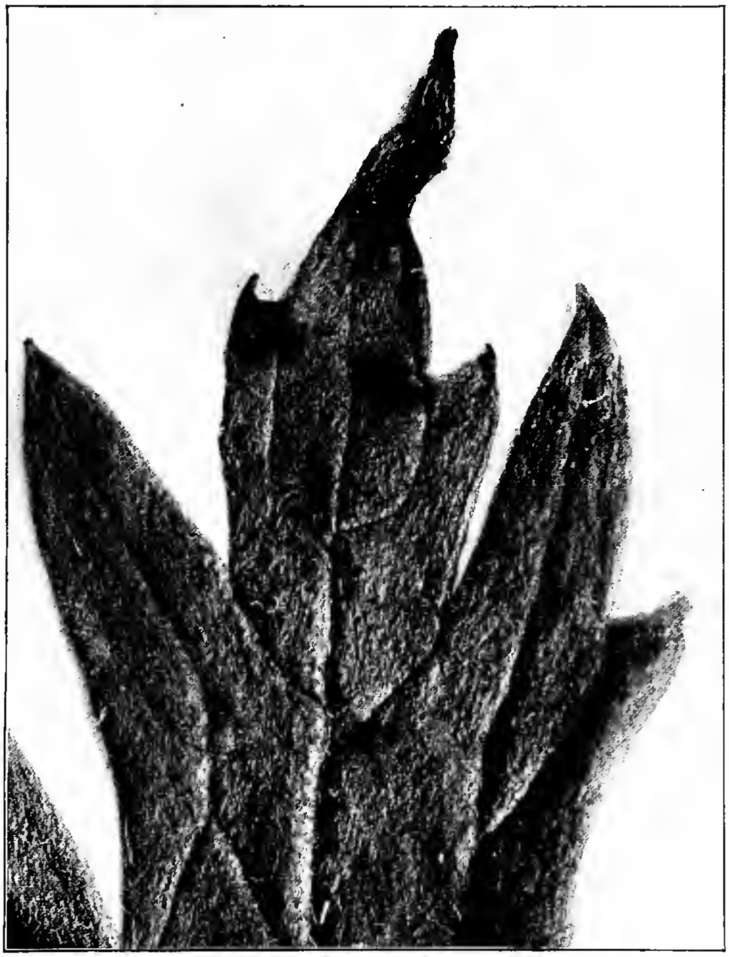

Fig. 127.*

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*Fig. 127.—Bacterial leaf-spot of the larkspur (Delphinium). Same as fig. 126, but photographed on Cramer's isochromatic slow plate. In this photograph the black spots on a green background come out distinctly; in fig. 126 they do not.

is desired. When using oil-immersion objectives see that there are no air-bubbles or particles of dirt in the cedar oil. The image on the ground glass should be observed the last thing before introducing the plate-holder, to see that it is free from images of objects not actually embedded in the slide. For the same reason slides and covers for mounting objects to be photographed must be cleaned with great care and kept clean until ready for use. Many really beautiful sections are ruined for photomicrographic purposes by having been mounted in dirty balsam or on dusty slides, or by being covered with soiled cover-slips. Sections should be cut and mounted in dust-free air, and the balsam used in mounting must be free from dirt. Much balsam on the market is very dirty and totally unfit for mounting sections designed to be photographed.

Glass surfaces through which it is designed to pass light should not be touched by the hands, greasy or otherwise. This applies to slides, covers, objectives, condensers, ray-filters, photographic lenses, mirror surfaces, ground glasses, negatives, lantern-slides, and what not. The least touch of the finger on a polished glass surface generally leaves its mark.

In using the common achromatic objectives for making photomicrographs, the "focus-difference" must be taken into account. Such objectives, being corrected only for two portions of the spectrum, require a different focus for the sensitive plate than for the human eye. In other words, an image which is perfectly sharp to the eye is not sharp for the sensitive plate and will yield a negative which is out of focus. By turning the fine adjustment a measured distance the image becomes hazy on the ground glass, but will then yield a sharp negative. A few exposures will determine just how much and in which direction the eye focus must be thrown out to give the sharpest result. The focus-difference may also be disposed of by using monochromatic light. The writer uses such light almost altogether, even with the best objectives. Another defect of achromatic objectives, and to some extent of all objectives, is an arching field, the center being out when the edges are in sharp focus. For this reason it is customary to select a small portion in the center of the field, make this as sharp as possible, and neglect the margins, which may be trimmed off on the print. The Spencer 16-millimeter apochromatic objective has the flattest field of any objective of like quality known to the writer. Lack of depth of focus is a serious defect in photomicrographic work, and must be compensated for by making the sections uniformly thin and mounting them perfectly flat. The student should read Sternberg in English and Neuhauss in German (Bibliog., LV).

For most stained sections involving bacteria, isochromatic plates are to be preferred, and slow rather than rapid ones. Exposure should be for contrast, and consequently as short as will give the necessary detail in the heavily stained parts. Development should be rather long and with an effort to obtain good contrasts. The writer formerly used hydrochinon, but now uses pyro, and develops until the image is visible on the back.

For general photographic work ortol is an excellent developer, and its preparation is extremely simple. If one uses the Hauff mixtures sold by Gennert, of New

York, all that is necessary is to dissolve one package of A in 20 ounces of distilled water and one package of B in an equal volume of water in another jar. For normal exposure on 5 × 7 plates, add 3 ounces of A to 3 ounces of B and dilute with 2 ounces of water. The picture begins to appear in thirty to forty seconds and development is completed in three to four minutes. To soften the harsh contrasts of underexposed plates or plates overexposed in parts, give a longer development, using 3 ounces of the alkaline solution to 1 ounce or ½ ounce of ortol and 4 or 5 of water. In the middle of a long development it is often important to change to a fresh portion of the developing solution. For overexposed plates, reverse the proportions, using 1 ounce or ½ ounce of alkali and 3 ounces of the ortol solution with several ounces of water. The advantages of this developer are its quick action and its freedom from stain and tendency to fog. The mixed developer may be used over and over until exhausted (browned). The quantity named above will suffice for a dozen 5 × 7 plates properly exposed. This developer may also be used with Velox paper. In this case it should be diluted with more water, say 1 ounce of the ortol solution, 1 ounce of the alkali, 6 ounces of water, and 6 drops of 10 per cent potassium-bromide water.

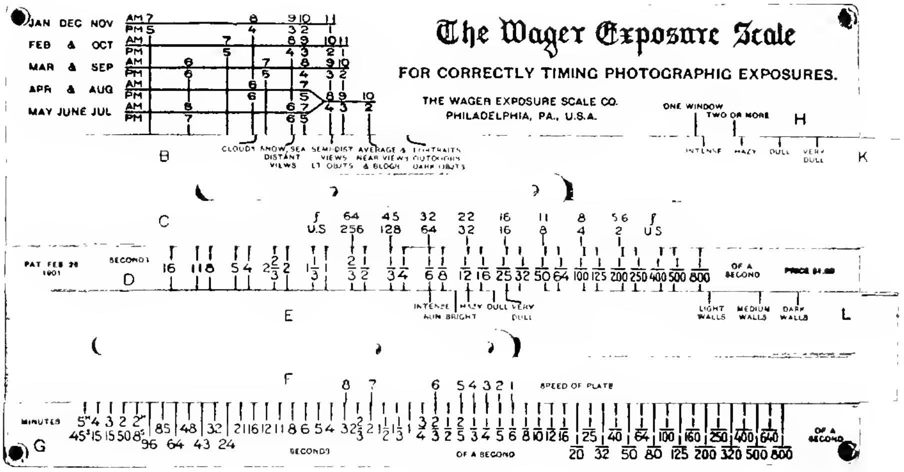

Fig. 128.*

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*Fig. 128.—Exposure scale set to show proper time for buildings and average near views at 10 a.m. to 2 p.m. in July, with stop 64 (32 f) and an intense sun. The various makes of plates are divided into eight classes, and the time is read from the middle scale for intense sun and the most rapid plates. Under above conditions a Seed's 27-X plate, or its equivalent (1), would require one-sixth second. For light of a less degree of brightness E is set on the proper stop, and the time is read from the bottom scale. The latter scale (G) is also used for slow plates. With intense sun, *i. e.*, as set above, a Cramer's isochromatic slow plate, or its equivalent (7), would require 2 seconds. In indoor work, scale K is first set on H, according to the quality of the light and number of windows. Scale L (kind of walls) is then set on the proper stop, and the time is read from the bottom scale, according to the speed of the plate used. In latitudes far to the north of Philadelphia there must be considerable increase of time, and there must be a corresponding shortening of time in tropical regions or desert regions. Considerable judgment must also be used in making indoor exposures, especially toward sunset and soon after sunrise. Near sunset, exposures have to be increased enormously. About three-fourths actual size.

Previous to development the exposed plate should be placed in the tray, flooded with water, and gently rubbed with the balls of the fingers, particularly if the exposures have been made for some time, or in dusty weather, or on plates which have been opened for some time. Many "pin holes" will be avoided by this practice, and frequently one will be astonished at the amount of dust which can be felt as the fingers are passed over the plate.

Negatives should be fixed in strong hypo for ten minutes (a little longer exposure will not harm them), hardened in alum-water (saturated) five or ten minutes if the weather is hot, and washed in running water one to two hours. If these rules are followed, negatives which are good on the start will not spoil afterward. Weak hypo should not be used, neither should the solution be saturated, but only nearly so, *i. e.*, a saturated solution diluted with one-sixth water. This is made up in small quantities in advance. The saturation is accomplished, not by throwing the crystals into a jar containing water, but by putting them into a cloth-sack which is brought into contact only with the top layers of the water. On removal from the washing-box the back and face of the negative should be rubbed over carefully under running tap water with a wad of soft cotton, and set away in a clean place to dry after rinsing in distilled water. If one is in a great hurry to get a print from a wet negative, it may be dried in about ten minutes by soaking for eight minutes in 95 per cent alcohol and then holding it near an electric fan.

In developing in deserts or in southern climates, in very hot weather, all the fluids must be iced, including the wash-water, or else the plate must be hardened in 2 per cent formalin water for five minutes before the development begins. Alum-water can not be used for this purpose, since it greatly retards development.

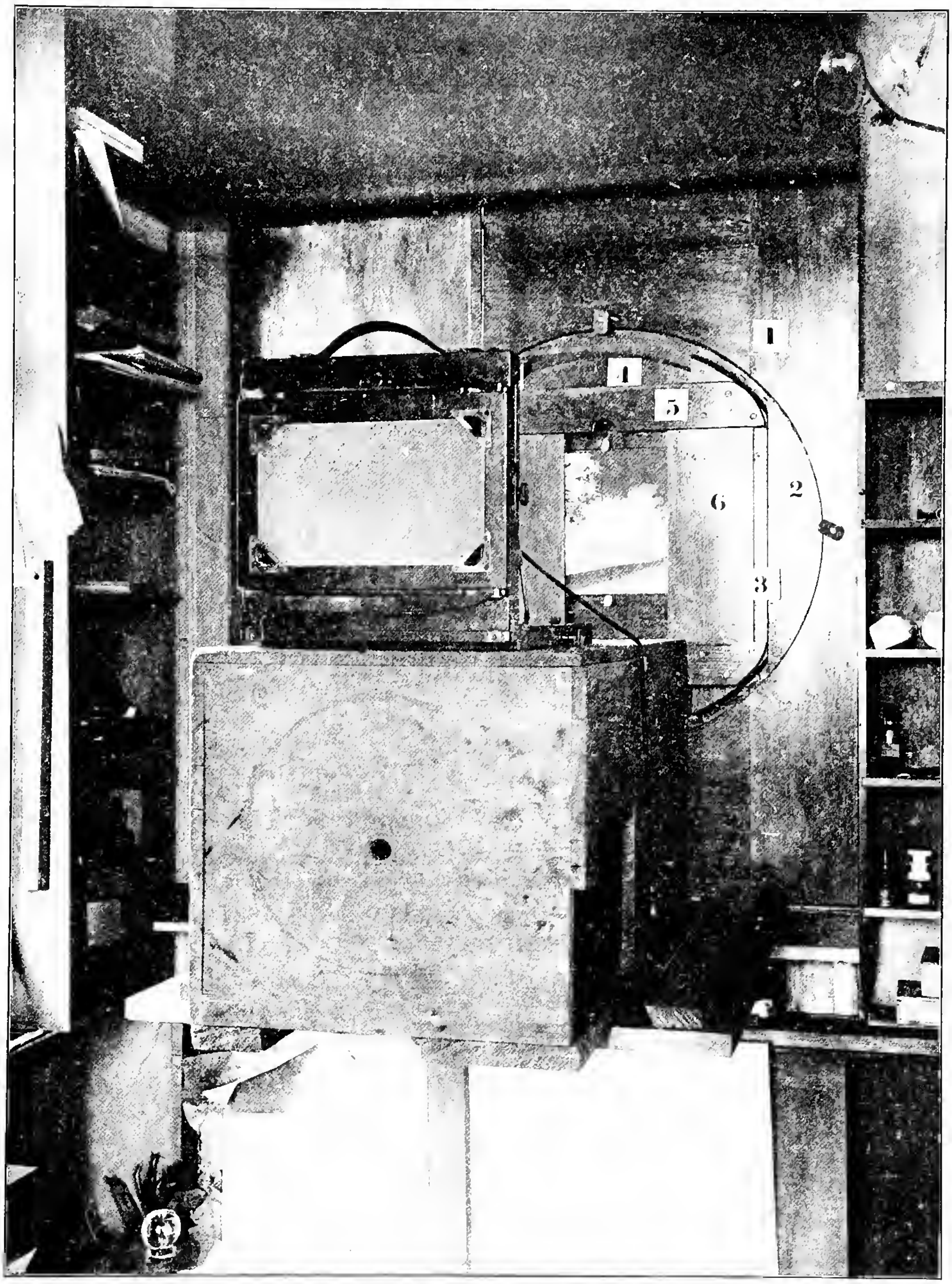
It often happens, especially with beginners, that a good negative (one rightly exposed) is spoiled by being left in the developing solution too long or by being taken out too soon. An overdeveloped negative may be reduced after soaking it in water (or preferably before it has dried) by placing it for a few minutes in a tray of clean water, to which has been added a small quantity of hyposulphite of soda and a few drops of a 10 per cent solution of red prussiate of potash (Farmer's reducing solution), which, of course, must be uniformly distributed. Thin negatives, free from hypo, may be intensified, if they are thin simply from underdevelopment, by exposure for from two to five minutes (occasionally a little longer) in a strengthening solution made of Agfa intensifier 20 parts and water 180 parts, or by soaking them in a strong watery solution of mercuric chloride until they are whitened through uniformly on the back, and then blacking them by soaking in ammonia water strong enough to give off disagreeable fumes. If the time of exposure is not nearly correct, another negative should be made. Negatives thin from overexposure do not intensify well; neither do those which were much underexposed. All negatives should have the subject, date of making, and degree of magnification written on them with a lead pencil as soon as they are dry. The proper place for a record is on the margin of the negative itself rather than in a book or on a bag, which may become misplaced, although it is convenient to have it also on the envelope, or negative-bag.

The correct time of exposure for photomicrographs varies so greatly with the size of stop, length of bellows, kind of slide, number of objective, quality of light, rapidity of plate, etc., that no very definite rules can be laid down, the right time in special cases in Washington varying all the way from several minutes to $\frac{1}{100}$ of a second. If the bellows-length is doubled, of course the time of exposure must be quadrupled. Low powers, and especially Planars, let through a great flood of light and require correspondingly short exposures. With low powers and sunlight the student might begin on $\frac{1}{25}$ second. With an oil-immersion lens and bright light he might try $\frac{1}{2}$ second or $\frac{1}{5}$ second. If the section is densely stained, much allowance must be made for that. It is well, at least for a time, to keep a record book of subjects and exposures to refresh one's memory. It saves the spoiling of many plates. Such a record should include subject, length of exposure, stop used, objective and eyepiece used, length of bellows, distance of the condensing lens from the Abbe condenser, time of day, time of year, quality of light, kind of screen, kind of stain and density of section, kind of plate, developer used, time required for development, and quality of negative, viz, overexposed, underexposed, or correctly timed.

For outdoor work, and also for natural-size or slightly magnified indoor work, a good exposure scale is sometimes useful. The best ones known to the writer are the Wynne and the Wager. Success with the Wynne depends on one's judgment as to the proper changes in a good sensitive paper; with the Wager it depends on one's judgment as to the quality of the light in the sky. After a little experience very uniform and excellent results may be obtained with either. Personally, the writer prefers to use the Wager (fig. 128), because it is simpler and takes less time. No scale is always to be depended on, there are so many variations in light and so many unprovided-for contingencies. Experience is after all the best guide, but until one has obtained it, genuine aids are not to be neglected. The beginner should first become familiar with the right exposure for one stop and one kind of plate, *e. g.*, stop f. 16 and Seed's 27, with a given bellows length. Having learned correct exposures under these constant conditions, it will be comparatively easy to change to other makes of plates and to other f. stops. Slow isochromatic plates require 10 to 12 times as long exposure as fast plates. In the matter of stops the length of exposure is, of course, quadrupled every time the f. stop number is doubled, and quartered every time it is halved, *e. g.*, if stop 16 will give a perfect negative with one second exposure, stop 8 will require one-fourth second and stop 32, four seconds. Under the same conditions, stop 4 will require one-sixteenth second, and stop 64 sixteen seconds, and so on. With the Universal stops (those commonly used on the shutters made in this country and England) the exposure is doubled for the next higher stop and halved for the next lower one, instead of quadrupled or quartered, as in the case of the f. stops.

For lantern slides the writer converts a small room into a camera box (plate 18). This room has a floor space about 6 by 5 feet. It has a north window and a west window. Each window is provided with a double set of roller curtains, the outer made of yellow cloth, the inner of a very dense black cloth known in the trade as double-faced, opaque, black shade-cloth, which lets scarcely any light through, even when held directly toward the sun. A cross-bar is screwed across the base of the uprights of the window frame, 35 inches from the floor and a few inches above the window sill. To this bar a swing shelf is hinged and drops down out of the way when not in use. This shelf is about 24 inches wide by 30 inches long. When in use it is supported in a horizontal position by a removable leg. On top of this shelf is placed a cracker-box or some similar box, to the sides of which, at the bottom, beveled cleats are nailed, which slide through corresponding cleats screwed to the top of the shelf. This enables one to push the box toward the window or draw it back on a regular track. On the top of this box, at the back end, or farthest point from the north window, the camera is placed facing this window and is screwed fast to the top of the box the same as to a tripod. Sidewise movement is provided by extending the screw-hole in the top of the box into a slot 6 or 8 inches long. In sliding the camera sidewise it is of course necessary to keep the ground glass parallel to the negative in the window, and this is done by drawing parallel lines on top of the box about $\frac{1}{8}$ inch apart and exactly at right angles to the negative-carrier. In moving the camera sidewise all that then remains is to see that one side of the camera at front and back matches one of these ruled lines. This gives ample sidewise movement, and some up-and-down movement is usually provided in the camera itself. The rest is obtained by moving the negative. The upper half of the north window is covered by the curtains. The lower part is filled with a removable wooden framework, the negative-carrier, so arranged that the negative itself may be moved up and down or sidewise, or twisted around at will. The framework of the negative-carrier is made of inch stuff. When in use it is placed upright about 3 inches in front of the window pane and just behind the cross-bar which keeps it in place. In the middle of this frame is a circular wooden disc (which must turn freely), held in place on the back by a ledge and in front by four buttons, and open in the center. The breadth of this disc is 24 inches, and it should be made of well-seasoned lumber of a sort not inclined to warp. On this disc at either side two broad vertical cleats are fastened. These are grooved on the inner edges next the framework, and under them, close to the circular piece, two wide $\frac{3}{8}$-inch pieces slide up and down freely, carrying the negative between them. The latter fits into marginal grooves and is held in place by buttons. The marginal grooves extend the whole length of the $\frac{3}{8}$-inch pieces and consequently allow the negative to be moved sidewise to any extent desired, while the up-and-down movement is obtained by sliding the two $\frac{3}{8}$-inch pieces and the negative between them as a unit. Over the first grooved cleats, at right angles, *i. e.*, horizontally, two similar cleats are screwed. These also have wide $\frac{3}{8}$-inch pieces moving under their grooved edges. These sliding pieces cover the sides of the negative and shut out the side-light in whatever position the negative may be placed. Behind the negative against the window is pasted (by its corners) a good-sized piece of white tissue-paper, which serves to distribute the light evenly and to cut out images of trees, buildings, etc. When in use the double curtains of the west window are drawn down and the door is shut. The north light which enters the room then comes through the negative placed in front of the camera. The focus is obtained

PLATE 18.



Small room arranged for making lantern slides and enlargements on bromide paper.

The parts of the window shutter are as follows: (1) frame work, (2) circular piece giving rotary motion, (3) one of two stationary pieces under which No. 4 slides, (4) negative carrier, (5) one of two stationary pieces under which No. 6 slides, (6) side pieces designed to cut out all the side light except that which comes through the negative.

on the ground glass as for any picture, remembering that a wide margin (½ inch or more) must be left for binding strips, and that if the negative has any up and down its image must be placed crosswise on the lantern-slide. The writer focuses as

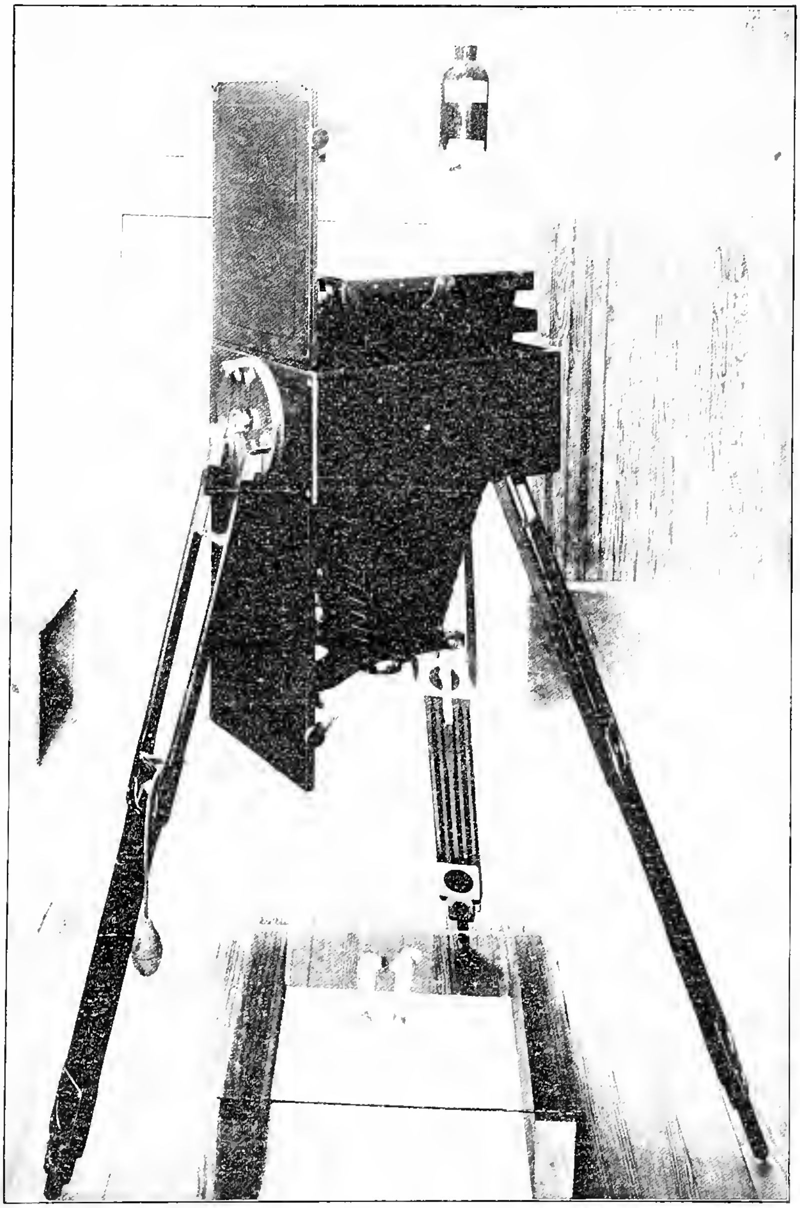


Fig. 129.*

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*FIG. 129.—The modified Collins-Brown camera used with tripod for natural-size pictures. Heavy shadows are dissipated by using the glass plate. The size of the camera box is 10¼ by 12¾ by 5¾ inches, and its weight, including lens and shutter, is about 15 pounds, or with tripod 19 pounds. The camera takes a 6½ by 8½ plate. It is solidly constructed, of the very best workmanship, and the only objection is its weight, which is no disadvantage in laboratory use. It is not recommended for field use.

sharp as possible with stop wide open and then stops down to 16 u. s. before making the picture.

There are two other ways of making lantern-slides, *i. e.*, by contact exposure, the gelatin films face to face, and by means of a long box-camera with the negative in one end, the lantern-slide carrier in the other end, and the lens between the two, *i. e.*, inside the camera-box, held in a framework sliding between the two ends and having front and rear bellows attached to its outside parts. The method by contact exposure is not very satisfactory unless the negative and the lantern-slide are of the same size. The box-method is a very good one. A box of this kind is very convenient, and may also be used for enlargements up to 5 or 6 times. The bellows-extension should be ample, so that various lenses may be accommodated and so that lantern-slides may be made from large negatives if desired, *i. e.*, the solid framework or track on which the parts slide should be about 6 feet long, and the bellows-extension to either side of the middle piece should be not less than 3 feet, exclusive of the woodwork at each end and in the middle. A very good apparatus of this sort is shown in plate 17. It is the Folmer & Schwing enlarging, reducing, and copying camera, mounted on a plain wooden table of home construction, and the only defect I have discovered in it is that it has too short a bellows for use with lenses having a 12-inch focus. It has a very neat device for obtaining a sharp focus and many other conveniences, and might just as well be made with a longer bellows. It is convenient to have a box which will take 11 by 14 plates. When making lantern-slides the end of the box carrying the negative is pointed toward the window and is elevated a foot or more to secure uniform lighting. The writer has found the Voigtlaender collinear lens, series III, No. 6, very satisfactory for making lantern-slides and enlargements. In plate 17 the bellows-extension used when making lantern-slides from large negatives lies on the floor.

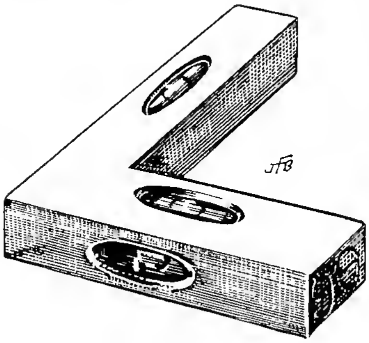

Fig. 130.*

The time of exposure for lantern-slides varies greatly with quality of light and density of negatives, *e. g.*, with stop 64 u. s. from ½ second or less in bright light to fifteen minutes or more in very dull light with dense negatives. Lantern-slides should not be developed with pyro because it stains, and should not be developed with metol-hydro because it often gives a foggy appearance if the contrasts in the negative are great or the exposure is a little too long. Hydrochinon gives very satisfactory slides.

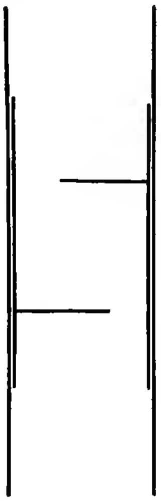

Fig. 131.†

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*Fig. 130.—Cross-level, made by The L. S. Starrett Company, Athol, Mass. Nearly actual size. This is very convenient for use with cameras.

†Fig. 131.—Device for cutting out light in air-shaft of dark-room. Diameter, 12 inches.

In lantern-slides one desires much detail and little density; it is customary, therefore, to develop only until there is a good surface image. On no account must the development be pushed until the image shows through on the back. Even the densest portions must be translucent. Slides suitable for projection with very bright lights may prove too dense for dull ones. In making lantern-slides one must keep in mind the kind of light to be used in projection.

In making enlargements, the camera is lowered to the horizontal and pointed away from the window, and the object is lighted in part from above (skylight). To facilitate shifts it is convenient to have the table-top (to which the camera-bed is screwed) turn freely on a central pivot, and the legs of the table should be mounted on casters so that it may be moved about easily. The table devised by the writer is shown in plate 17. This was built by a carpenter and does well enough. In making enlargements the lens-board is removed from the interior and substituted for the kits in the front end of the camera; the ends of the wooden carrier (shown on first shelf of the camera-table) are slid under the beveled cleats at the front end

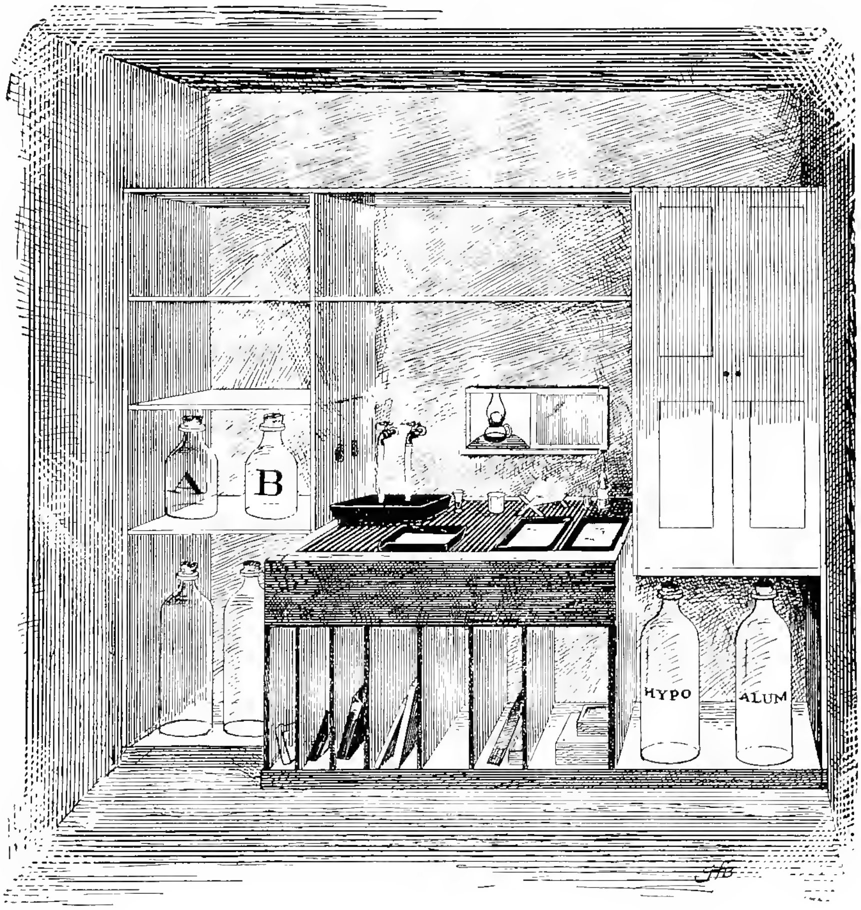

Fig. 132.*

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*Fig. 132.—Side view of a convenient small dark-room, devised by Mr. Hubbard.

of the table, and the map or other object to be enlarged is then pinned on a flat board in the right position in front of the lens, the board being held in place by the carrier. The desired magnification is obtained by sliding the carrier in or out to a marked place previously determined.

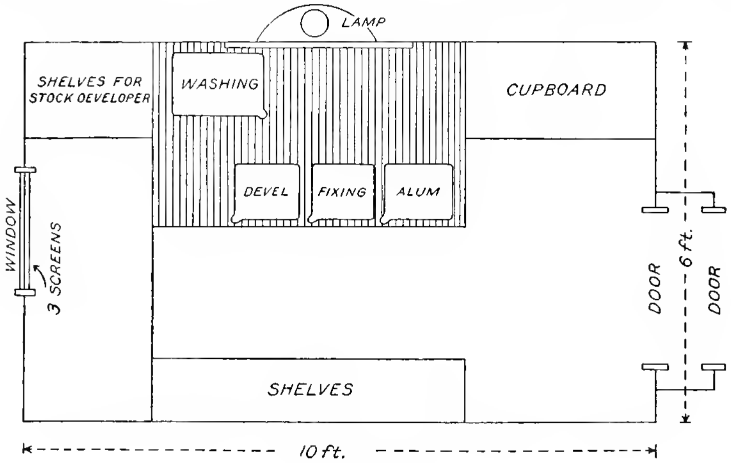

Fig. 133.*

When not made directly from the microscope, the histological drawings in this book have been made from photographic enlargements. For example, in fig. 72 a solio print or bromide print was made from the photomicrograph. This was then enlarged three or four times and from the resulting negative a salted-paper silver print or a blue print was made. A drawing was then made on this print with a fine-pointed pen and waterproof India ink. After careful inspection by the writer and such changes as were required to make the drawing correspond more nearly in all its details to the main lines of the photograph, the brown of the silver, or the blue of the iron salt, was removed by a bath in water containing cyanide of potash. On reduction by the photoengraver many of the inequalities in the pen-work of such drawings disappear and the pictures closely resemble the originals, whereas if they are drawn without enlargement, and engraved as drawn, the pen lines will in many cases have a more or less ragged appearance.

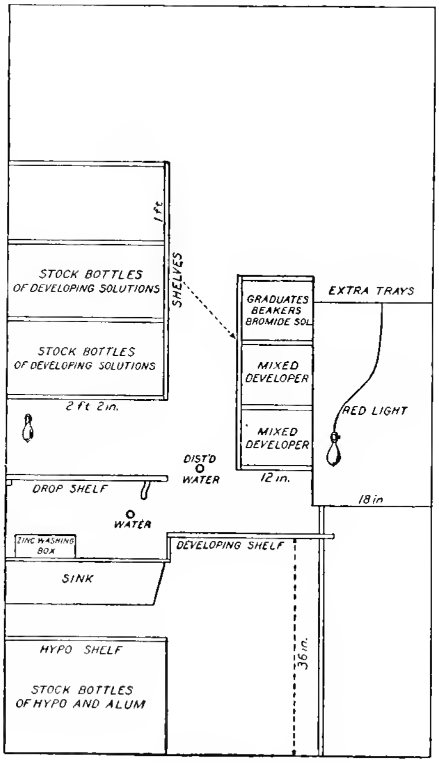

Fig. 134.†

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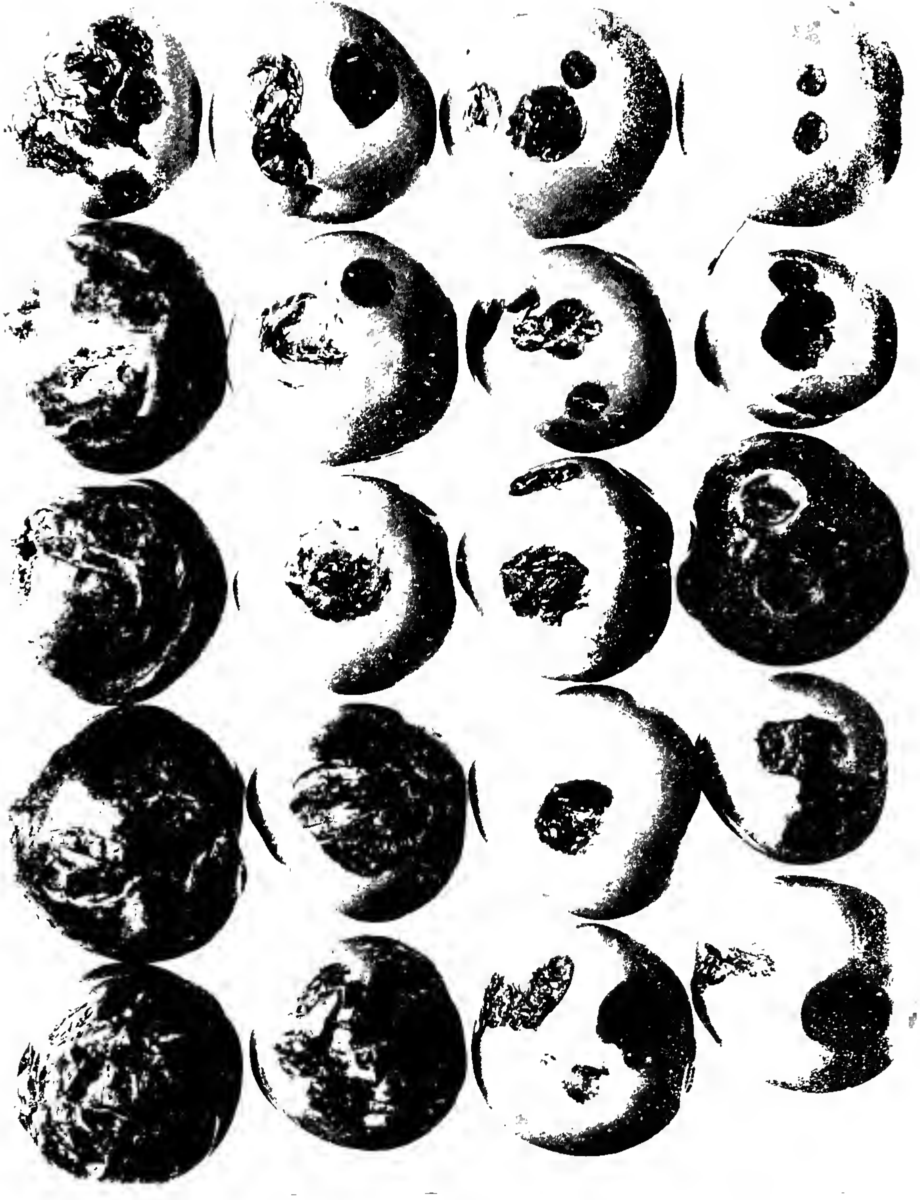
*Fig. 133.—Top view of the room shown in fig. 132. All of the trays rest on triangular slats covering a deep sink. The screens are raised and lowered very easily by balanced weights.

†Fig. 134.—Side view of a small photographic dark-room in Laboratory of Plant Pathology.



Bacterial black spot of the plum.

Spots about six weeks old, except a very few on the two right-hand plums of the upper row. Fruits about one-half grown; collected July 24, 1902, from an orchard of Japanese plums in central Michigan. In this stage the plums (var. Hale) begin to crack open and are attacked occasionally by fungi (Monilia, etc.). Some tufts of Monilia may be seen on the outer, left-hand fruit, second row from the bottom. Early stages of this disease are shown in figs. 70, 71, and 72.

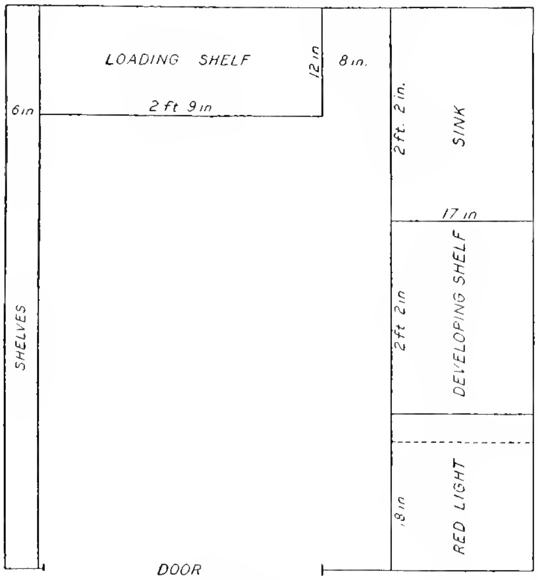


Fig. 135.*

The very convenient heavy camera shown in fig. 124 may also be used for natural-size work, arranged as shown in fig. 129. In this connection the Starrett cross-level shown in fig. 130 will be found very convenient for leveling the back of the camera.

Very excellent cameras are made by the Century Camera Company. Their Long-focus Century Grand leaves little to be desired in the way of a convenient, perfect-working instrument.

The dark-room for development is an important subject. The chamber must be light-tight. At the same time it ought to be roomy and well ventilated. If the room is small and in use much of the time, some means of removing the foul air becomes imperative. The writer accomplishes this by an electric fan placed in the mouth of an air-shaft which extends from the ceiling to 6 or 8 feet above the roof. These shafts are cylindrical, 1 foot in diameter, made of heavy sheet-iron and surmounted by a broad, mushroom-shaped cap. The interior is painted a dead black, and as an additional precaution against the entrance of light it carries a sleeve of the form shown in fig. 131. This effectually cuts out light. The air is pumped out so rapidly by a device of this sort that not the least inconvenience is experienced in working all day in a very small room.

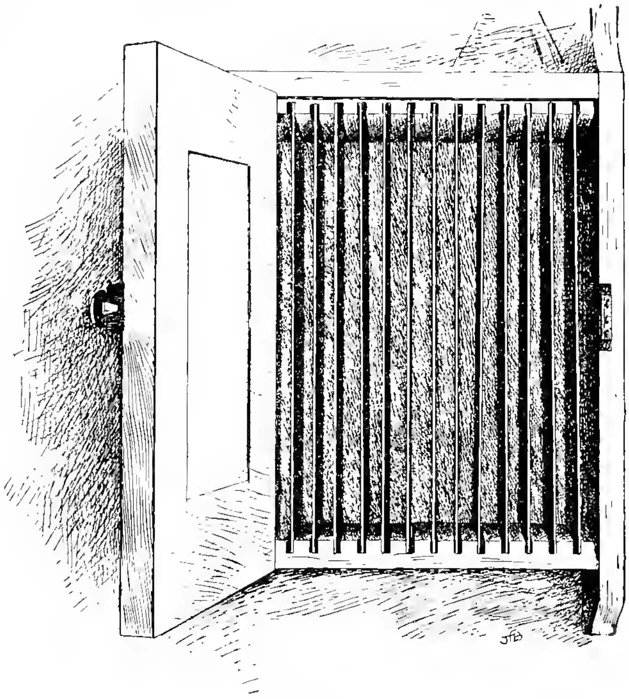


Fig. 136.†

If only one or two persons

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*Fig. 135.—Diagram of small dark-room shown in fig. 134. Standing in the middle, a man can touch the walls in either direction. In the ceiling is a foot-wide pipe extending 6 feet beyond the roof and capped with a broad mushroom top. In the lower end of this pipe is an electric fan, which pumps foul air out of the room. Light is prevented from entering by partial cross-septa projecting from opposite sides of the air-shaft, and also by blacking its inner surface.

†Fig. 136.—Wall case for preserving from dust and scratches the enameled iron plates used for squeegeeing silver prints.

of good habits are to use the dark-room, it is very convenient to have developing dishes and fixing trays on the same shelf, which may be of slats over a deep sink, as in figs. 132 and 133. With some shelves over this sink and a water-tap above it, everything is in reach without moving about. If, on the contrary, various persons are to work in the dark-room, some of them students with unformed habits, some of them older workers with incorrigibly slovenly habits, including a disposition to spill hypo over everything, then some different arrangement must be made, the sodium hyposulphite trays especially being kept on a separate shelf at a good distance from the developing shelf. Figs. 134 and 135 show the arrangement of a small dark-room devised by the writer for photographic work, the space at his disposal being very limited. The air-shaft is in the ceiling over the loading shelf. Artificial light is furnished by two 16-candle power Edison electric-light bulbs, one hanging near the wall above the sink, the other inclosed in the red-light box. Over the ruby glass there is placed a sheet of orange-buff paper, commonly called post-office paper. The hypo trays are under the sink. The zinc box for washing negatives stands in the sink. Developers are mixed on the drop-shelf, and are kept on the shelves above it. The bromide bottle, graduates, and beakers are kept on the small shelves above the developing shelf. Large bottles of alum solution, hypo solution, etc., are stored under the hypo shelf.

Enameled plates for squeegeeing silver prints may be stored when not in use as shown in fig. 136. In this way they are protected from dust and scratches. To prevent prints from sticking the surface of the plates is occasionally rubbed with paraffin dissolved in xylol, and is then polished with a soft clean cloth.

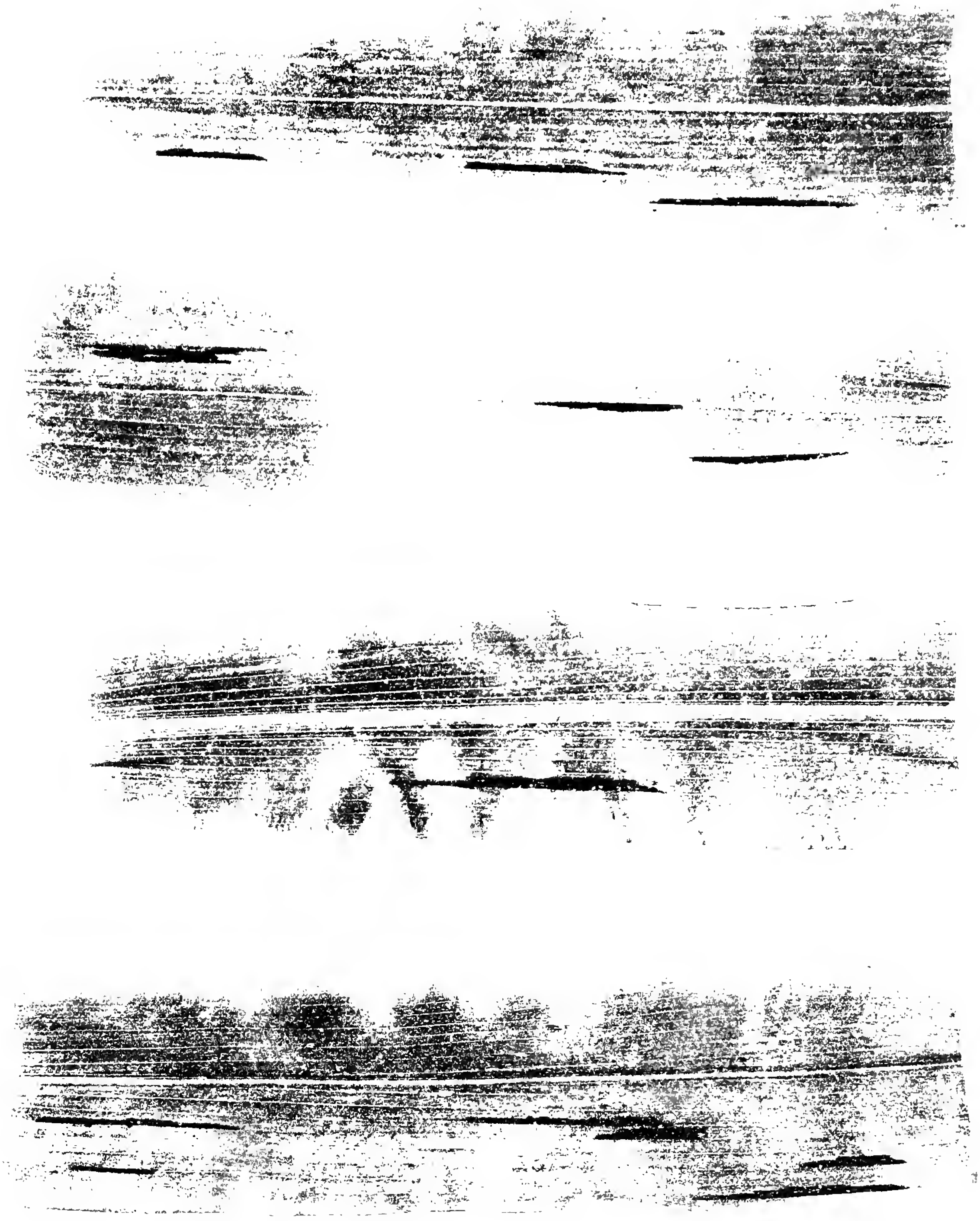
Some memoranda on photographic developers will be found under *Formulæ*. In addition to what has been said there a few hints on salted silver-paper, blue-print paper, and bromide-papers may be of service to those who wish to use these methods as preliminary to pen-and-ink drawing.

All papers designed for this use should possess a smooth surface suitable for pen-and-ink work, and sensitive papers of this quality may be had of various dealers by specifying just what is desired. Blue-print paper and salted silver-paper may be made for one's self. It is preferable, however, to purchase the former, and to make the latter or to buy it fresh, as it does not keep well. Directions for making the plain silver-paper may be found in "The Figures, Facts, and Formulæ of Photography and Guide to their Practical Use," by H. S. Ward, N. Y., Tennant & Ward, 1903; and in "The Photo Miniature," Vol. II, No. 22, "Albumen and Plain Paper Printing."

All sensitive papers placed in the printing frame should have the coated side next to the film-side of the negative.

Blue-print paper is much less sensitive to light than solio paper, and long solar printings are required. When the paper has assumed a deep-bronzy appearance it may be assumed to be sufficiently printed. A few trials will give the necessary experience. Blue-prints are developed by simply washing them in several changes of pure water, taking care that the coated surface is wetted thoroughly from the start, *i. e.*, freed from air-bubbles. No fixing is required.

PLATE 20.



Bacterial disease of broomcorn. (Hothouse, U. S. Department of Agriculture, spring of 1905.)

Disease not complicated by aphides. The signs consist of gradually elongating red or red-brown stripes on a green background. In later stages the stripes coalesce and the leaves shrivel. Under surface of diseased areas often covered by red crusts—the dried-down, bacterial ooze from interior of leaves. The infections were by way of the stomata and these spots are about six weeks old.

Salted silver-paper also requires a rather long printing with sunlight. When properly printed the paper is washed in a bath of salt-water, rinsed in several changes of pure water, and fixed in a weak solution of hypo (1 : 15).

Bromide-prints under the negative are usually made by exposing the paper to artificial light at a standard distance, say 9 inches or 12 inches. By always exposing at a given distance and to a light of uniform intensity two variable factors are excluded, and one then has to take into account only the quality of the paper and the density of the negative. It is usually economical, especially for beginners, to test the density of the negative in advance by exposing, for various periods, narrow strips of the sensitive paper laid across the negative so as to include dense and thin portions. These strips are then developed, and if none of them have been properly exposed, a second trial is made. When the right exposure has been learned, the print is made. A little experience enables one to judge quite correctly as to the proper exposure by simply looking through a negative. No very definite rules can be given for length of exposure; this depends so much on distance from the light and brightness of the flame. With velox paper and an ordinary flat gas-flame at a distance of 9 inches the writer's negatives usually require from 15 seconds to 2 minutes. With a Welsbach light or with thin negatives the time would be shorter. At a distance of 18 inches from the light the time would, of course, be quadrupled. Directions for the employment of special developers usually accompany each maker's paper. The writer has found ortol (p. 141) a very good developer for velox paper, and prefers it to metol-hydro. Velox-prints are developed in weak artificial light (gas turned low); they are rinsed from the developing solution by passing quickly through a bath of pure water; they are then fixed in hypo, and washed for at least one-half hour in running water. The writer pins the prints to a smooth board and floats these in a bath-tub or clean sink, film-side down. Most of the curling may be avoided by drying the prints, film-side down, on mosquito-netting stretched on a wooden-frame.

The yellowing of prints is often due to the fact that they were not properly fixed; the hypo solution was weak, or the time of exposure to it was not sufficient.

All pen-and-ink drawings on such photographic prints must be made with waterproof India ink, after which the photographic part is bleached out by exposure for a few minutes in water containing cyanide of potash (1 : 500, more or less). The drawings should be exposed in this bath only as long as necessary. If any part of the print refuses to bleach, it should be moistened with iodine potassium iodide and returned to the cyanide bath. It is then passed through pure water and dried face up on blotting paper in a place free from dust.

SOME MILESTONES IN THE PROGRESS OF BACTERIOLOGY.

The development of bacteriology can not be separated from advances in human and animal pathology. Physicians and surgeons have made most of the brilliant discoveries or have led the way to them. Chemists and physicists have assisted. With a few shining exceptions, botanists have had comparatively little to do with the advancement of this science. Bacteriological inquiry has been an incentive to the improvement of various kinds of apparatus, notably the microscope, and it has derived corresponding advantages from the use of these improved instruments of research. We owe, in particular, a great debt to the German physicist Abbe, whose discovery of the Jena glass made possible the superb modern apochromatic objective.

Among the multitude of workers in animal pathology and bacteriology during the last thirty-five years certain men tower far above the rest, their contributions to science having been more conspicuous and their imprint on their generation more lasting. If France is mentioned, we think at once of Pasteur, Davaine, Duclaux, Metchnikoff, Chamberland, Roux, Nocard, and Chauveau. In Germany we think of Virchow, Cohn, Cohnheim, Koch, Weigert, Nicolaier, Eberth, Gaffky, Hueppe, Flügge, Fraenkel, Pfeiffer, Behring, Ehrlich, and many others; in Japan, of Kitasato and Shiga; in the United States, of Welch, Sternberg, Theobald Smith, Nuttall, Councilman, and a host of brilliant younger men, many of whom received their training under Welch in the Johns Hopkins Pathological Laboratory. England, from which one might have expected so much, has contributed comparatively little, owing probably to the laws in force in that country respecting animal experimentation, laws framed with the intention of doing a kindness to the lower animals, but working, on account of their interference with the pathologist, a distinct detriment both to men and animals, the aim of all animal pathological inquiry being the alleviation of human and animal suffering. In passing we should not forget, however, the contributions of Tyndall and Lister, the one a physicist, the other a surgeon.

Undoubtedly bacteriology owes very much to Louis Pasteur. France has had many great sons, none greater than he. His refutation of the doctrine of spontaneous generation cleared the air of many misconceptions and laid the foundations for exact experimentation. His demonstration of the nature of pébrine and flacherie, two destructive diseases of silk worms, brought again into vivid light the assumption that the origin of a great variety of human and animal diseases should be sought in the activities of microscopic organisms. His studies of anthrax and other diseases of warm-blooded animals confirmed this suspicion and set a great many persons thinking and working. His investigations of the problems connected with fermentation were similarly fertile in discovery and in suggestion.

The publication of Robert Koch's great paper on tuberculosis in 1884 marked another distinct advance. The same memorable year Koch published in full his discovery of the cause of Asiatic cholera, only a brief announcement of it having been made in 1883. The whole world was interested, and from this time on experimenters began to multiply in every civilized land, boards of health, universities, and private citizens vying with each other in the establishment of laboratories for the study of these minute organisms endowed with so much power for good or evil. Koch's investigations in South Africa bring us down to recent times, where the perspective is not so good. To sum up very briefly, omitting many things, the following are some of the milestones:

1. Overthrow of the doctrine of spontaneous generation.
2. Discovery that putrescible fluids (exclusive of milk) will not decay after boiling, if protected from the bacteria of the air by means of cotton-plugs.

3. Pasteur's studies of fermentations; discovery of anaerobic organisms.

4. Pasteur's studies of pébrine, flacherie, anthrax, chicken-cholera, and rabies.

5. Cohn's system of classification.

6. Cohn's discovery of endospore-bearing organisms resistant to heat.

7. Introduction of anilin stains and photomicrography.

8. Tyndall's discontinuous moist sterilization.

9. Lister's antiseptic surgery.

10. Lister's dilution method for obtaining pure cultures. Ascribed also to Naegeli.

11. Miquel's discovery of thermophilic bacteria.

12. Discovery of root-tubercles of Leguminosæ by Woronin, and subsequent papers by Beyerinck, Hellriegel & Wilfarth, *et al.*

13. Discovery of bacterial diseases in plants by Burrill, Prillieux, and Wakker.

14. General introduction of Koch's poured-plate method for obtaining pure cultures.

15. Koch's discovery of the "comma bacillus," the cause of Asiatic cholera.

16. Paper on tuberculosis by Koch in Mitth. a. d. Kaiserlichen Gesundheitsamt, Bd. II.

17. Use of synthetic media, of pressure filters, of fermentation tubes, and of other anaerobic apparatus.

18. Introduction of apochromatic objectives.

19. Eberth and Gaffky's discovery of the bacillus of typhoid fever; Nicolaier's discovery of the tetanus bacillus; Loeffler & Schutz's discovery of the bacillus of glanders; Salmon & Smith's discovery of the hog-cholera bacillus; Yersin's and Kitasato's independent discovery of the bacillus of plague; Pfeiffer's discovery of the organism causing influenza; Shiga's discovery of the cause of tropical dysentery.

20. Winogradsky's studies of nitrifying organisms.

21. Hansen's studies of fermentation, more especially yeasts.

22. Duclaux's, Greene's, and Brown & Morris's study of enzymic actions.

23. Study of toxines and anti-toxines; general use of anti-diphtheritic serum.

24. Migula's attempt to determine the exact morphology of all known species.

25. Discovery of the cause of peripneumonia in cattle by Nocard & Roux; organism so minute as to be at the extreme limit of microscopic definition.

26. Profound specialization, resulting in distinct classes of bacteriologists, *e. g.*, animal and plant bacteriologists, milk bacteriologists, water bacteriologists, soil bacteriologists; and in special societies and journals, *e. g.*, those devoted exclusively to the study of tuberculosis.

Beyond this field, but of extreme pathological interest, and worked out by the exact methods of the bacteriologist, are Laveran's discovery of the protozoan causing malarial fevers and Theobald Smith's discovery of the protozoan causing the bovine disease known as Texas fever, both parasites of the red blood-corpuscles. More recently it has developed that these are not rare types of disease. On the contrary, many virulent diseases of man and the lower animals are now known or believed to be due to Tripanosomes or other Protozoans, and the literature on the subject is becoming voluminous.

NOMENCLATURE AND CLASSIFICATIONS.

The nomenclature of the bacteria is in a somewhat chaotic state, as might be expected of a science which has been cultivated so largely by medical men and so comparatively little by systematic botanists and zoologists. The writer therefore will venture a few remarks on this subject.

If an organism is distinct from any which has been described, so as to be regarded as a new species or spoken about as a distinct thing, then it should be given a specific Latin name and not designated by a figure or a letter of the alphabet. Bacillus No. 1, 2, or 3, or A, B, C, is proper enough for private memoranda while an investigation is incomplete, but when it is finished and ready for publication these designations should give place to scientific names.

Naturalists everywhere are in agreement that the scientific name of a living thing should consist of two words only—the name of the genus, followed by the name of the species, after which is usually added the name of the author, or, if a transposition has been made from one genus to another, the name of the original describer is put into a parenthesis, followed by that of the transferrer outside of the parenthesis. All polynomials, of which there are now many, are to be regarded as *nomina excludenda*. For example, *Bacillus coli communis* should give place to *B. coli*, and such names as *Bacillus membranaceus amethystinus mobilis*, *Bacillus argenteo phosphorescens liquefaciens*, *Bacillus pyogenes foetidus liquefaciens*, should yield to something shorter and more in conformity with modern views of nomenclature. More than 170 trinomial names are to be found in the last edition of Flügge's Mikroorganismen, and very few, if any, of them were given with the distinct idea that they represent varieties of other organisms. The habit of giving trinomial or quadrinomial names should be abandoned, and as far as possible binomial names should be substituted for those already in literature.

In the period antedating Koch's discovery of the poured-plate method, when there was no very satisfactory way of separating one organism from another so as to have pure cultures, the descriptions were necessarily vague. They were usually drawn from mixed cultures, and very brief descriptions were supposed to be ample. The result is that many of the names which have come down from this period are *nomina nuda*, or semi-nuda, *i. e.*, it is impossible to associate them with any known organism for the very good reason that they were not founded on any one organism, but on mixtures now indeterminable, or were too imperfectly characterized. Generally speaking, such names should be abandoned. The only safe rule and the only just one is to discard all specific names which do not carry with them an exact statement or description, sufficient to associate the name beyond doubt with a particular organism. It is not sufficient description of an organism to say that it is the cause of a disease, unless the author has proved it to be such according to the well-recognized rules of pathology. In order that his name shall hold, an author must have carefully described the disease and must have *proved* in some way the pathogenic nature of his organism, or else he must have given a fairly correct description of the morphology and physiology (cultural characters) of the organism, so that it can be detected anywhere. Just how much shall constitute a sufficient description must depend on circumstances. A few lines might be sufficient if the description were exact and of such a character as to definitely indicate a particular organism as the one intended. Many pages would be insufficient if the description is vague and contradictory and does not enable the scientific public to fix upon a particular organism as the one intended. The careful work of subsequent investigators may sometimes lead an author to say that he meant to designate such or such organisms by his names, but if he really described something different or made no intelligible descriptions, then his names can only be regarded as equivalent to *nomina nuda* and should never be substituted for later ones given after careful study and description of the organism. Any other course puts a premium on bad work. In case of the higher plants and animals, preserved specimens will often serve to correct a faulty description and to indicate clearly *the object* to which the name was applied. Cultures of particular bacteria kept alive by means of frequent transfers to fresh culture-media will also serve the same purpose when they are able to run the gauntlet of extermination by other organisms accidentally introduced during some one of the many transfers, and when they have not varied too greatly from the original type as a result of changed environment, but dead and dry organisms, in most cases, offer only a most dubious and uncertain means of identification. Who, for example, would undertake to determine what is included under the name *Bacterium termo* in von Thuemen's dried collection, No. 1000? The name *Bacterium gummis* affords a good example of what the writer has in mind. *Bacillus vascularum solani*, *Bacillus caulivorus*, *Bacillus gossypina*, and *Micrococcus pellucidus* are also examples of names given unaccompanied by any proper description of the organism. Many additional ones might be cited. There is no lack. To found, for example, a new species of rabbit on the observation that a small jumping animal about the size and shape of a rabbit had congregated in certain turnip fields and caused great damage and apparently had destroyed no other plants would only serve to provoke a smile or to raise a doubt as to the author's mental condition, and yet descriptions equally worthless are not at all uncommon in systematic bacteriology. The *Micrococcus pellucidus*, although published quite recently and in the Comptes Rendus of the French Academy, is not described any better. "I find it quite impossible," says Mr. Stoddert, "to identify many species from published descriptions." Numerous complaints of this sort, made in recent years by well-trained and competent men, sufficiently indicate the necessity of a thoroughgoing reform.

Various more or less arbitrary dates have been assumed by zoologists and botanists as the proper beginning of species priority, none of which can be used in bacteriology. In the opinion of the writer the only proper starting point is from the time when bacteriologists were first able to make and easily maintain pure cultures of any given organism, namely, from the discovery of the poured-plate method of isolation in the year 1881. Nearly all species characterization prior to this time is a cloudland of uncertainty, and while it may be possible fifty or a hundred years from now, when the whole field of bacteriology, as we now understand it, shall have been thoroughly worked over, to decide by the doctrine of exclusion, with some degree of probability, what was meant by certain old names, nothing whatever can to-day be made out of the description accompanying these names. And here I wish to register a protest against anything of this nature ever being done. If, in his own generation, a name can not be associated beyond doubt with a particular organism by means of an author's description or figures or collected specimens, then this name should disappear, never to be revived. Societies of bacteriologists should unite in the near future on some authoritative date for the beginning of species priority, so that some sort of stability may be guaranteed to the nomenclature of the future.

In the way of generic nomenclature there is not much of value prior to Cohn's first great paper in the year 1872. It seems perhaps rather commonplace reading now, but it really marked a great advance and was the result of twenty years of diligent inquiry. Inasmuch as there is no present agreement among bacteriologists as to the limits of common genera, the same genus name being used with very different meanings by different writers, it appears worth while to discuss the subject of genera at some length.

At the outset three principal inquiries arise. First, what character or congeries of characters shall be considered of generic value; second, what generic names shall be used; third, what meaning shall be attached to these names?

In the description of species it is necessary to draw very largely upon physiological characters, but it will not be disputed, I think (certainly not by naturalists), that genera ought to be founded, if possible, entirely upon morphological characters, in conformity with the usages of other branches of natural history. Physiological characteristics may be used to help out our description of sub-generic groups, such as the yellow *Bacterium* (*Pseudomonas*) group, the green-fluorescent *Bacterium* (*Pseudomonas*) group, the hog-cholera group, the hay-bacillus group, the *Proteus* group, the *Tyrothrix* group, the *Urobacillus* group, etc., but morphology appears to be sufficient to distinguish the genera.

Quite dissimilar organisms are still put by many writers under the same genus name, but the tendency to carefully discriminate is on the increase, and before many years, it is safe to say, writers on bacteria will be using generic names with a definite morphological meaning. Certain it is that we can not go on much longer calling any rod-shaped organism Bacillus or Bacterium interchangeably, or putting it into the one genus or the other according as we happen or do not happen to find it producing endospores, or growing as a short rod or as a filament. Some light may come from considering with what meaning such generic terms were originally used. Matters would also be much simplified by accepting 1872, the date of the appearance of Cohn's first great paper, as the proper date for the beginning of generic nomenclature of the bacteria, using only such earlier names as he accepted, emending his conception of these genera in such ways as experience has shown to be necessary, and adding new names from time to time as new genera are discovered. If some date is not settled upon in the near future, then we may expect an attempt to substitute certain names which have not been at all used for the last thirty years, *i. e.*, since bacteriology became a science, for those which are now in common use. The confusion which would result from an attempt of this sort and the utter uselessness of making such a change are sufficient grounds for desiring an authoritative expression of opinion on the part of organized bodies of men cultivating this branch of science before we are precipitated into any such confusion. The question raised is this: Shall we abandon modern generic names given to definite, well-known, and easily recognizable organisms for old names given before there was any science of bacteriology, not now in use, and most of which can not be attached with certainty to any specific organism, or to any definite group of organisms?

From time to time, as new discoveries have been made, our views as to what should be considered generic characters have undergone decided modifications, as everyone knows who is familiar with the various writings on systematic bacteriology, especially those which have been the most widely read and have exerted the most influence. A full discussion of all the various problems of generic nomenclature is not, however, contemplated in this connection. It is safe to predict that no system now extant can be looked upon as a finality, since we know as yet too little about these numerous and variable organisms to devise an altogether consistent system. Classifications are conveniences, nothing more. Some conform more nearly than others to the observed facts, but none are perfect or, from the nature of the case, can ever be final. What the future may have in store no one can tell. There will undoubtedly be many surprising discoveries, and recent attempts at classification may then appear very crude. Our concern, however, is chiefly with the present and with knowledge as it appears to-day.

On the whole, the classification of Migula, which was proposed in October, 1894, and is outlined at length and applied to most of the well-recognized forms, in his beautiful great work, "System der Bakterien," appeals to me most strongly. Up to this time the writer has followed this system in his own publications and will continue to do so, with certain modifications, until some distinctly better system makes its appearance. This system is based on the flagella and is much more workable than one based on spores, or on a combination of these two characters. The presence or absence of flagella and their position on the body are used by Migula as generic characters.

In 1895 Dr. Alfred Fischer also propounded a new system of classification based on spores and flagella. This system was republished in 1897, with material modifications, in his "Vorlesungen über Bakterien," and is modified still further in the second edition of that work. In the non-twisted, rod-shaped bacteria use is made of the flagella to separate the subfamilies, while the generic characters are derived from certain phenomena incident to spore-formation. The following table of 17 genera, taken from his first paper, shows this system at a glance:

FISCHER'S TABELLARISCHE UEBERSICHT DER BACILLACEEN.

Sub-families.	Flagella.	Shape of rods containing endospores.			Arthrospores.
		Cylindric.	Spindle-form.	Clavate.	
Bacilleĭ.....	None......	Bacillus.....	Paracloster..	Paraplectrum...	Arthrobacter.
Bactrinieĭ...	One polar flagellum.	Bactrinium..	Clostrinium..	Plectrinium.....	Arthrobactrinium.
Bactrilleĭ...	Polar flagella tuft.	Bactrillum...	Clostrillum..	Plectrillum.....	Arthrobactrillum.
Bactridieĭ...	Diffuse flagella.	Bactridium..	Clostridium..	Plectridium Diplectridium.	Arthrobactridium.

Concerning this classification it should be pointed out that large groups of bacteria are omitted altogether, namely, those which produce neither endospores nor arthrospores. This, so far as we yet know, includes nearly all the plant parasites. About one-half of the genera were hypothetical at the time the paper was published, *i. e.*, not founded on any organism, as I have already pointed out in another place.* The question of whether an endospore-bearing rod is or is not swollen around the spore is often difficult to determine, and as Migula and Lehmann & Neumann have pointed out, the endospore-bearing rods in some species may be either cylindric or spindle-form, or bear the spores in the middle or at one end. The whole question of the existence of arthrospores is still a matter of doubt. Closely-related forms, and even the same species, may possess one or several polar flagella. The genus Bacillus was founded by Cohn on *Bacillus subtilis*, which is now known to have peritrichiate flagella, *Bacillus ulna*, also actively motile, and *B. anthracis*, which is non-motile. Inasmuch as Cohn's studies were made chiefly on *B. subtilis*, he having never studied *B. anthracis*, but only including it as a sort of afterthought, for the sake of completeness, and because *Bacillus subtilis* is the first one described, it seems only proper that the term *Bacillus* should be restricted to motile forms resembling the hay bacillus, *i. e.*, those having diffuse flagella, and should not be transferred to the non-motile forms. For the hay bacillus and similar forms Dr. Fischer has used the name Bactridium. This name, however, is inadmissible because preoccupied and that, too, whether bacteria be considered as plants or animals. Bactridium has been used as a genus name seven times, as follows:

Bactridium Kunze, 1817: For fungi, 11 species of which are recognized in Saccardo's Sylloge Fungorum.

Bactridium Salisb., used in 1839 as a sectional name under Erica (DC. Prod.), and also in 1889 by Drude in Engler & Prantl's Die Natür. Pflanzenfamilien. It is said by Baillon to be a synonym of Syringodea Benth. Bentham reduced Don's genus Syringodea to a section of Erica.

Bactridium LeConte, 1861: Col., p. 86 MS.; Bactridium Sauss., 1863, Orthop. M. Scudder: Genera in Zoology.

Bacteridium Davaine, 1868: For the organism causing anthrax.

Bactridium Schroeter, 1872: For *Micrococcus prodigiosus* and various other pigment-bearing bacteria, most of which have since been included under Bacillus.

Bactridium Fischer, 1895: For *Bacillus subtilis*, *B. megaterium*, *B. typhosus*, etc.; *B. typhosus*, however, being non-sporiferous, so far as known, has logically no place in Fischer's original classification, as already pointed out, since the mere fact of the absence of endospores does not presuppose the existence of arthrospores. The same remark applies to *Bacillus amylovorus* and to many other species.

Dr. Fischer himself knew of the existence of Kunze's genus *Bactridium*, and refers to it, but he does not appear to have known of Davaine's use of the word for the anthrax organism. He thinks that Kunze's "rare, little-known fungi" are so different that there will be no confusion, and insists on using the word with an entirely different meaning for the most curious of all reasons, viz, "um die Harmonie der Nomenclature nicht zu stören." The only possible ground on which such use could be defended is that bacteria are so different from plants and animals that duplication of generic names is not a matter of any consequence. If this were so, then he should, nevertheless, according to all recognized rules of nomenclature, have used the word with a different concept, since the first use of Bacteridium in this group was to designate a non-motile organism. Davaine used this word long ago in a perfectly plain and legitimate way to separate the non-motile from the motile forms, and the organism, which he studied most carefully as the type of his new genus, is that which was only some years afterwards designated *Bacillus anthracis*, namely, in 1872, when Cohn formed his new genus Bacillus. If the genus Bacteridium is to stand at all as a bacterial genus, it must be used for *Bacillus anthracis* and its congeners, and it is an eminently proper name for these organisms, provided bacteriologists can bring themselves to think, with Fischer, that this name is not too close to the fungous genus *Bactridium* Kunze on the one hand or to the animal genera on the other, which have priority. The writer does not share this opinion. There would seem to be also an older name than Dr. Fischer's *Plectrinium* for monotrichiate bacteria having the spore borne in a swollen end, viz, Trécul's *Urocephalum*. This, according to Trécul, was a motile bacterium coloring deep blue with iodine. Trécul describes it as 0.02 mm. long, "à queue flexueuse," with distinct spore formation at one end which was enlarged. The subfamily name Bactrideï is also open to objection because preoccupied. There is a genus of palms, Bactris, and in 1889 Drude, in "Die Natürlichen Pflanzenfamilien," used the subtribal name Bactrideae, which is the same word as Bactrideï.

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*Review in Am. Naturalist.

Dr. Fischer has not helped matters by the modifications introduced into his "Vorlesungen" as the result of criticism, since he has destroyed the logical consistency of his system by including sporiferous and non-sporiferous forms under the same genus name.

MIGULA'S CLASSIFICATION OF THE BACTERIA.

The bacteria are phycochrom-free schizomycetous plants with division in one, two, or three directions of space; reproduction by vegetative multiplication. Resting stages in the form of endospores are produced in many sorts. Motility due to flagella occurs in some genera. In Beggiatoa and Spirochaeta the organs of motion are unknown.

I. Order EUBACTERIA.

Cells without any "Centralkörper" and free from sulphur and bacteriopurpurin; colorless or faintly colored, also chlorophyll-green.

I. Family COCCACEAE Zopf emend. Mig.

Cells globose in a free state; in stages of division often somewhat elliptical. Division in one, two, or three directions of space without previous elongation of cell. If the cells remain united after division, they are frequently flattened at points of cohesion. In all Coccaceae with cells large enough for observation septation takes place in the globose state before there is any elongation perpendicular to plane of division. Only a few species are motile.

Streptococcus Billroth.

Cells globose and without organs of motion. Division in only one direction of space. When the cells remain united after division, moniliform chains are produced, or diplococcus forms, but the latter also occur in other genera of Coccaceae. Doubtful if any spore formation.

Micrococcus (Hallier) Cohn.

Cells globose in a free state. Division in two directions of space without previous elongation of cell. No organs of motion. Endospore formation not positively demonstrated and probably wanting. When the cells remain together after division Merismopaedia-like plates may be formed, in which case the contiguous cell-walls may be flattened.

Sarcina Goodsir.

Cells globose, in a free state. Division in three directions perpendicular to each other. No organs of motion. Spore formation doubtful. If the cells remain united after division, bale-like constricted packets are formed; frequently these do not appear, as the nutrient medium has the greatest influence on the form of the cell-unions.

Planococcus Migula.

Cells globose but usually adhering in twos or fours with points of contact flattened. Division in two directions of space, as in Micrococcus. Motile by means of one or two long, wavy-bent flagella. Spore formation unknown.

Planosarcina Migula.

Free cells globose. Division in three directions of space, as in Sarcina. Motile by means of long or short flagella. Apparently only one flagellum to each cell. No endospores. Usually the cells remain united after division as diplococci or tetracocci, but seldom in the form of packets.

2. Family BACTERIACEAE.

Cells longer or shorter cylindric, straight, or at least never spirally twisted. Division in one direction only, viz, perpendicular to long axis, and only after a preliminary elongation of the rod. In some species the rods separate early; in others they remain united for a considerable time as longer or shorter threads. A single cell, so far as known, does not immediately break up into two daughter cells when the first septum is formed, but remains as a single rod until additional septa are laid down. In some species the cells may be very short, so as to superficially resemble Streptococci, but an exact study of the cell-division enables one to distinguish with certainty.

Bacterium Ehrenberg (char. emend.).

Cells cylindric, longer or shorter, often forming threads of considerable length. Without organs of motion. Endospore formation occurs in many species, but appears to be entirely wanting in others. In many they may yet be discovered when the organisms are exposed to suitable environments.

Bacillus Cohn (char. emend.).

Cells straight, rod-shaped to ovoid, longer or shorter, sometimes united into quite long threads. Motile by means of wavy-bent flagella which are scattered over the whole body. Endospore formation frequent. In most species motility occurs only during a definite period of development, which is very brief in some species and very long in others.

Pseudomonas Migula.

Cells cylindric, shorter or longer, sometimes forming threads. Motile by means of polar flagella. The number of flagella on a pole varies from 1 to 10; most frequently it is 1, or 3 to 6. Endospore formation certainly occurs in some sorts, but is rare.

A separation into two genera does not appear to be desirable at the present time. No such difference exists inside this genus as there is between the genus Microspira, which only exceptionally has more than one flagellum on the pole, and the genus Spirillum, which has many polar flagella. Between the one-flagellate and many-flagellate forms there are all sorts of transitions in the genus Pseudomonas. Possibly the boundary between Pseudomonas and Microspira is artificial. Slight crooking of the rods, especially in stained preparations, has been observed in many species of Pseudomonas, and it is not always possible to decide whether a one-flagellate form belongs to this genus or to Microspira. Ordinarily, a decision may be reached by observing the shape of the threads or chains, those of Pseudomonas never being twisted into the form of a screw.

3. Family SPIRILLACEAE (Screwbacteria).

Cells spirally wound or representing a portion of the turn of a spiral, in which latter case the entire spiral is visible only when several cells remain united. Endospore formation established for some species, but rare. Apparently, in some of the larger species resting forms are also produced by the breaking up of the rods into short segments which envelop themselves in a gelatinous membrane. Usually motile. Division only in one direction of space perpendicular to the long axis.

Spirosoma Migula.

Cells generally rather large, spirally bent, rigid, and without organs of motion. Cells single, free, or united into small gelatinous families.

(1) Subgenus EUSPIROSOMA: Cells single or united into a spirally twisted thread. Free, that is, not inclosed in any gelatinous envelope.

(2) Subgenus MYCONOSTOC: Cells single or united into spiral threads, which are surrounded by a roundish general envelope.

Microspira Schroter.

Cells bent like a comma or sausage, rigid, single or several united, in which latter case screws or S-shaped figures are produced. Motile by means of 1 wavy-bent polar flagellum (rarely 2 or 3 flagella). The flagellum is usually not much longer than the cell. Endospore formation has thus far not been demonstrated. Many authors do not distinguish between this genus and the next.

Spirillum Ehrenberg.

Cells rigid, rods of various thicknesses, length, and pitch of the spiral, forming either long screws or only portions of a turn. Cells motile by means of a tuft of polar flagella (5 to 20), which are mostly half circular, rarely wavy-bent. These flagella occur on one or both poles. Their number varies greatly and is difficult to determine, since in stained preparations several are often united into a common strand. Endospore formation has been observed in some species. There are many undescribed species.

Spirochaeta Ehrenberg.

Cells thin, mostly quite long, motile and flexible, winding snake-like, but also moving in the manner of a screw. Organs of motion unknown. Endospore formation not observed.

Nearly related to the Algal genus Spirulina, but colorless and not segmented into single cells.

4. Family CHLAMYDOBACTERIACEAE.

Cells cylindric, united into threads which are surrounded by a sheath. Reproduction by means of motile or non-motile conidia which arise directly from the vegetative cells, and without passing through any resting stage grow into new threads.

Chlamydothrix Migula.

Cells cylindric, non-motile, arranged in unbranched threads, with a sheath of varying thickness. Frequently the septation of the threads is only demonstrable after the use of reagents. Reproduction by means of non-motile, roundish or ovoid conidia, which arise directly from the vegetative cells. Syn.: Streptothrix (Cohn) Mig., Leptothrix Kütz. exp., and Gallionella Ehrenberg exp.

Crenothrix Cohn.

Cells united into unbranched filaments, attached, and gradually enlarging toward the free end, *i. e.*, with a distinction between base and apex. Sheath rather thick. In iron waters the old and empty sheaths are permeated by ironoxidhydrate. Cells cylindric or flat discoidal. Multiplication by non-motile (mostly roundish) conidia, which arise from the vegetative cells by division and rounding off. For this purpose the cells of the thicker threads divide in three directions of space, those of the thinner threads only perpendicularly to the long axis of the thread. The conidia are discharged and germinate immediately, often on the sheath of the mother-thread. Only one species known.

Phragmidiothrix Engler.

Cells cylindric, later discoidal, forming threads 100 μ long and 3 to 12 μ thick, with a very delicate, scarcely visible sheath. Multiplication by non-motile conidia, which arise from the vegetative cell by division in three directions of space and rounding off. Perhaps to be united with Crenothrix, if the projections observed by Engler are not branches but epiphytes. Only one sort known.

Sphaerotilus Kützing (1833).

Cells cylindric, enveloped in sheaths, forming dichotomously branched threads with no differentiation into base and apex. Multiplication by means of conidia, which swarm out of the sheath, attach themselves anywhere, and immediately grow out into new threads. The conidia possess a tuft of flagella inserted sidewise under one pole.*

Genera, the systematic position of which is doubtful: *Spiromonas* Perty; *Spirodiscus* Ehrenberg; *Achromatium* Schewiakoff; *Newskia* Famintzin; *Streblothrichia* Guignard.

II. Order THIOBACTERIA.

Cells without any "Centralkörper," but with sulphur inclusions. Colorless, or pigmented rose, red, or violet by bacteriopurpurin; never green.

1. Family BEGGIATOACEAE.

Filamentous bacteria, destitute of bacteriopurpurin. Division of cells in one direction of space, viz, perpendicular to the long axis.

Thiothrix Winogradsky.

Threads attached, not uniformly thick, enveloped in a delicate sheath which is not easily demonstrable, non-motile, contents containing sulphur granules. The threads produce rod-shaped conidia at their end. These conidia, which are self-motile by means of a slow, creeping motion, attach themselves by one end to any sort of substratum, extrude a slime-cushion at the base, bend over ordinarily in their middle to a nearly right angle and grow into a new thread. Habitat, hot sulphur springs.

Beggiatoa Trevisan.

Threads destitute of a sheath, formed of flat discoidal cells, free, *i. e.*, not attached. Multiplication by folding and separation of the threads. Motile by means of an

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*Streptothrix and Cladothrix are omitted from the second volume, the species, so far as they represent bacteria, being distributed in other genera. *C. dichotoma* becomes *Sphaerotilus dichotomus*.

undulating membrane, as in Oscillaria. The organism creeps along, but at the same time rotates around the long axis, mostly with a swinging of one or both free ends. Habitat, hot sulphur springs and other fluids in which hydrogen sulphide is developed. No reliable method is yet known for the separation of the species. The number and size of the included sulphur-granules are not of specific value. They depend on the amount of hydrogen sulphide in the water.

2. Family RHODOBACTERIACEAE.

Cell-contents rose, red, or violet, from the presence of bacteriopurpurin. Sulphur granules are also included.

Classification still very artificial, owing to imperfect knowledge. Author follows Winogradsky

(I) Subfamily THIOCAPSACEAE.

Cells united into families. Division of the cells in three directions of space.

Thiocystis Winogradsky.

Families small, compact, enveloped singly or several together in a gelatinous cyst, capable of swarming. When the families have reached a definite size they escape from the gelatinous cyst, the latter either swelling and softening uniformly or at some particular spot. The escaped cells either pass into the swarm stage or unite into a larger fused complex of families, the individual cells of which separate and swim away only after a long time, and by means of much vigorous struggling.

Thiocapsa Winogradsky.

Non-swarming, globose cells spread out upon the substratum in flat families, which are loosely enveloped in a common gelatin. The membrane is split by the growth of the family, and the cells are separated as if by the swelling of an intermediate substance.

Thiosarcina Winogradsky.

Non-swarming cells arranged in packet-shaped families, corresponding to the genus Sarcina in the Eubacteriaceae.

(II) Subfamily LAMPROCYSTACEAE.

Cells united into families. Divisions of the cells, first in three and then in two directions of space.

Lamprocystis Schroter.

Families at first solid, then globose-hollow, becoming perforated net-form; cells finally separating into small groups which are capable of swarming.

(III) Subfamily THIOPEDIACEAE.

Cells united into families. Divisions in two directions of space.

Thiopedia Winogradsky.

Families tabular, formed of cells arranged in fours and capable of swarming.

(IV) Subfamily AMOEBOBACTERIACEAE.

Cells united into families. Division in one direction of space.

Amoebobacter Winogradsky.

Cells connected by plasma threads. Families amœboid motile. The cell families slowly change form, the cells drawing together into a heap or spreading out widely, thus bringing about a change in the shape of the whole family. In a resting condition a common gelatin is extruded, the surface of which becomes a firm membrane.

Thiothece Winogradsky.

Families inclosed in a thick gelatinous cyst. Cells capable of swarming and very loosely embedded in a common gelatin. When the swarm stage supervenes, the cells lie more loosely, the gelatin is swollen, and the cells swarm out singly and rather irregularly.

Thiodictyon Winogradsky.

Families consisting of rod-shaped cells having their ends united into a net. Out of an originally compact mass of rods there gradually results from rearrangement a Hydrodictyon-like cell-union, which, under unfavorable conditions, may again draw together into a compact mass of rods. The multiplication of the families results from division or by loosening of slowly motile small cell-colonies.

Thiopolycoccus Winogradsky.

Families solid, non-motile, consisting of small cells closely pressed together. Multiplication of the colonies by the breaking up of the surface into numerous short shreds and lobes which continue to split up into smaller heaps.

(V) Subfamily CHROMATIACEAE.

Cells free, capable of swarming at any time.

Chromatium Perty.

Cells moderately thick, cylindric-elliptical or elliptical. Polar flagella.

Rhabdochromatium Winogradsky.

Cells rod-shaped or spindle-form, with flagella on the poles.

Thiospirillum.

Cells spirally twisted.

To the preceding may be added a third order, Myxobacteriaceæ, which is not discussed critically by Migula and is not adequately described in any of the text-books. The following general characters are taken from the papers of Dr. Roland Thaxter, to whom we owe our knowledge of these curious and interesting organisms. The most recent paper is by Erwin Baur, Myxobakterien-Studien, Archiv für Protistenkunde, V Bd., I Heft, pp. 92-121, 1 pl.

MYXOBACTERIACEAE.

Motile, rod-like organisms, multiplying by fission, secreting a gelatinous base, and forming pseudoplasmodium-like aggregations before passing into a more or less highly developed cyst-producing, resting state, in which the rods may become encysted in groups without modification, or may be converted into spore-masses. The vegetative rods, which vary little in size and form in the different genera and species, are typically elongate, sometimes reaching 15 μ in length. Cell-division follows an elongation and nearly medium constriction of the rods, which, except at the moment of division, are always separate; never united in chains. A slow, sliding locomotion and a Beggiatoa-like, bending motion is characteristic of the active rods. Organs of motion have not been detected. In all species, with one exception, the rods, when seen in masses, are more or less distinctly reddish. A distinct, firm, hyaline, gelatinous base is secreted by the colony as it extends itself, over which the individuals may move or in which they become embedded.

The vegetative period, in artificial cultures, usually lasts about a week, or even two weeks, but in nature the production of cysts must be more rapid. Common in moist situations on dung, decaying wood, fungi, lichens, etc. According to Bauer they grow best at 30° C.

In forms like Myxococcus, in which the rods are somewhat scattered, the first preparation for spore-production as seen under the microscope consists in the appearance of groups of rods moving with a circular tendency, in which the more central individuals soon become converted into spores. The formation of a cystophore, when it occurs, results from the basal constriction of a papillate mass of rods which projects from the surface of the colony. In the encysted condition there are two classes—one in which the individuals thus encysted show little or no modification from the rod-like vegetative state, the other in which they are converted into definite spores. They, however, seem to run into one another.

The species have been arranged under three genera, as follows:

Chondromyces B. & C.

Rods forming free cysts, in which they remain unmodified. Cysts various, sessile, or borne on a more or less highly developed cystophore.

Polyangium Lk. (Myxobacter and Cystobacter syn.).

Rods forming large, rounded cysts, one or more free within a gelatinous matrix raised above the substratum.

Myxococcus Thaxter.

Rods slender, swarming together after a vegetative period to form definite, more or less encysted, sessile or stalked masses of coccus-like spores.

That which appears least defensible in Migula's classification is his use of the word *Bacterium* for the anthrax organism and similar non-motile bacteria. If this generic name is to be retained, it should be used somewhat as Ehrenberg used it, viz, for motile organisms, and should not be given to entirely different non-motile forms. We have the right to set aside so much of Ehrenberg's description as does not correspond to facts, but not more. We do not know exactly what Ehrenberg had in mind, it is true, but it certainly was not non-motile forms of the type of the anthrax organism.

Provided one goes back of Cohn's time (1872), which the writer is not disposed to do in case of the bacteria, the one species by which the generic name Bacterium must stand or fall is *Bacterium triloculare* Ehrenberg. In size and shape, as described and figured, it agrees very well with some of the larger species of Pseudomonas Migula. If we can trust Ehrenberg's distinct statements and his plain figure, it was provided, like most species of Pseudomonas, with one polar flagellum. Ehrenberg also figures and describes it as trilocular or triarticulate. He may have been wrong in including it among his Vibrionides and in figuring and describing it as possessed of a polar flagellum, an organ difficult to make out in unstained material and with the crude microscopes in use in his day, but, while we bear this in mind, we must not forget that the person who was using these microscopes was no ordinary observer, but a man with remarkable eyesight and with a genius for observations of this kind. Moreover, the tri-locularity which he observed may have been simply the organism in early stages of division, which is the more likely, since he states that he saw it divide, and because in his specimens from Berlin he sometimes observed four septa and sometimes only two. Ehrenberg's description of the genus Bacterium, taken as a whole, is of course worthless for purposes of modern classification, our ideas of generic values being entirely different from his. A few things only come out of the rubbish heap of this early writing in a serviceable condition. The organism was a short rod, multiplying by cross-septation, possibly also by means of spores, colorless, sluggishly motile, moving, it is said, by means of one polar flagellum, and occurring abundantly in water containing rotten vegetation, where bacteria would be likely to abound. It should also be noted that it did not take up colored particles, such as indigo or carmine, when placed in water containing these substances. If the word Bacterium is used, it should be in conformity to these facts, or supposed facts, and may be so used, I think, until they are shown to be erroneous.

The matter is simplified, however, if we start with Cohn's use of the word Bacterium in the year 1872. The Bacterium of Cohn is a certain *Bacterium termo*. While we are not able to tie down Cohn's use of this name to a particular species, it appears that we can do it quite definitely to a group of morphologically similar species. Much discredit has been thrown on *Bacterium termo* in modern times, and it has been left out of many classifications. However, if one examines into the matter, there is no reasonable doubt as to what Cohn had in mind. His *Bacterium termo* was a small schizomycetous organism capable of growing freely in Cohn's nutrient solution, containing acid potassium phosphate and ammonium tartrate. It produced therein short rods (single, in pairs, or fours joined end to end) and roundish-lobed (kugelige-traubige) white zooglœæ, together with a *greenish fluorescence*. This is Cohn's statement and de Bary's. It did not appear in boiled fluids, *i. e.*, was destitute of endospores (Cohn), and the motile rods were killed by a short exposure to 58° C. (Schroeter). In other words, it was a non-sporiferous green-fluorescent organism possessed of a single polar flagellum, or, in some cases perhaps, provided with paired or triple polar flagella. If we start with Cohn's classification in the year 1872, we may keep the name *Bacterium* for schizomycetous organisms of this type, and at the same time we shall not be doing any violence to the older use of the word by Ehrenberg, who figures and describes this kind of an organism. This the writer proposes to do, substituting Bacterium (Cohn emend.) for Pseudomonas Migula and for more recent names proposed by others. Cohn's *description*, be it understood, is worthless for the most part, but his name Bacterium (*B. termo*) is usable *because it can be attached to a definite kind of organism.* To show that Cohn's use of this word and the writer's use of it do not conflict with former usage, Ehrenberg's descriptions and figures of Bacterium are here reproduced from the expensive and not readily accessible publications in which they appeared.

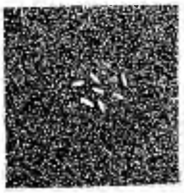

Fig. 137.*

The organism described as *Bacterium triloculare* by Ehrenberg is shown in figs. 137 and 138, and Ehrenberg's account is summarized as follows:

The genus Bacterium was founded by C. G. Ehrenberg in 1828, and was characterized by him in the Symbolæ Physicæ, Animalia evertebrata. The book in which this description occurs is an unpaged folio. On the second page of the text proper, in a list of species found "In Oasi Iovis Hammonis Siwae" this genus

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*Fig. 137.—*Bacterium triloculare.* From Ehrenberg's Symbolæ Physicæ. Animalia evertebrata. Decas prima. Berlin, 1828. Plate II, fig. 6.

is first mentioned as follows: "4. Bacterium *triloculare* n. g." The second mention of the name is on the fourth page in a list of "Genera et Species, in Europe a me nondum visa." The genus is described on the sixth page as follows:

> BACTERIUM, Novum Genus, Familia *Vibrioniorum*. Character Generis: Corpus polygastricum? anenterum? nudum, oblongum, fusiforme aut filiforme, rectum, monomorphum (contractione nunquam dilatatum), parum flexile (nec aperte undatum), transverse in multas partes sponte dividuum.
>
> *B. triloculare* nov. spec.: distincte triloculare s. triarticulatum, subfusiforme, hyalinum.
>
> Phytozoa Tab. II. Libyca fig. 6.
>
> Animalculum 1/300 lineae longum, corpore tereti. Articuli s. septa interna divisionem instantem multiplicem transversam indicare videntur. Mobile sed pigrum animalculum.
>
> In Oasi Iovis Hammonis Siwae observatum, praeterea nullibi.
>
> Bacterii Generis physiologia hucusque obscura. Cibo colorato ventriculos replere hae formae respuunt ideoque ad Polygastrica non nisi dubitanter et interim collocantur.
>
> Bacterium *simplex* vide *Monas simplex*. Bacterium *scintillans* vide *Monas scintillans*.

In general, Ehrenberg's figures are excellent where he could see anything to draw, but the seven figures of *B. triloculare* are very small (white on a dark-gray background), and one can make nothing out of them beyond the fact that they are minute bodies, 3 or 4 times as long as broad and with two indistinct septa. At the foot of the plate their length is said to be $\frac{1}{250}$ of a "linie." The figures are more or less rounded at the ends and show no constrictions at the septa.

In 1830, in his "Beiträge" (see Bibliog., V), Ehrenberg has the following on the genus *Bacterium:*

> Phytozoa. Classis I Polygastrica. A. Anentera. Ordo I Nuda. Family I Gymnica. Corpore non ciliato, oreciliato nudove (p. 37). Sectio II. VIBRIONIA. Elongata, in se nunquam contracta. Sub-section *c:* Corpore oblongo, fusiformi aut filiformi (tereti aut triquetro nec quadrangulo) aperte undatum non flexili, nec spirali:
>
> BACTERIUM. nov. Gen.—Haec genera, Oscillatories valde affinia, ore nutriri nondum vidi. 11 species (p. 38).

In another place the following species are mentioned, without description, as belonging to this new genus: *Bacterium cylindric*, *B. deses*, *B. Enchelys*, *B. fuscum*, *B. monas*, *B. punctum*, *B. termo*, *B. tremulans*. The number 11 was apparently completed by the three others, *B. triloculare*, *B. simplex*, and *B. scintillans*, not here mentioned. In this paper Ehrenberg made use of the subsequently oft-quoted expression: "Die Milchstrasse der Kleinsten Organization geht durch die Gattungen Monas, Vibrio, Bacterium, Bodo." This idea appears to have impressed him greatly, for he repeats it in his "Infusionsthierchen."

In 1832, in Ehrenberg's "Geographische Verbreitung," a paper which was read January 10, 1828, and written apparently before either of the preceding, but not printed until 1832, three species of *Bacterium* are mentioned, *B. simplex*, *B. triloculare*, *B. scintillans*, all as new species, but there is no description of the genus or reference to any description.

In 1832, Ehrenberg again returns to *Bacterium* in a paper, "Ueber die Entwickelung," etc. In this paper six species are included in the genus *Bacterium*, but four of them he now regarded as doubtful:

Gattung XII Bacterium E., Gliederstäbchen.
‡) deutliche Gliederung.
1. B. articulatum E., Perlenschnurgliederstäbchen.
[Descript.] Bewegung zitternd. Berlin.
2. B. triloculare H. und E., dreifächriges Gliederstäbchen.
‡‡) undeutliche Gliederung.
3. B. ? Enchelys E.
4. B. ? punctum E.
5. B. ? tremulans E.
6. B. ? termo E.

In 1838, in his magnificent work, "Die Infusionsthierchen," Ehrenberg gives additional information respecting the genus *Bacterium*. It is there described as follows:

Die quergetheilten gehören zu den Zitterthierchen (Vibrionien), den längsgetheilten zu den Stabthierchen (Bacillarien) (p. 2).

Vierte Familie: Zitterthierchen (Vibrionia, Vibrionides).

Character: Animalia filiformia, distincte aut verismiliter polygastrica, anentera, nuda, gymnica, corpore Monadinorum uniformi, divisione spontanea imperfecta (transversa), catenatim consociata, hinc filiformia.

Charactère: Animaux filiformes, distinctement ou vraisemblablement polygastrique, sans canal alimentaire, sans carapace, sans appendices, à corps uniforme des Monadines, se réunissant par division spontanée imparfaite (transversale) en chaines filiformes (p. 73).

Zitterthierchen sind Monadinen, welche, durch queere unvollkommene selbsttheilung, bewegte Gliederfäden bilden (p. 74).

This family contained five genera: Bacterium, Vibrio, Spirochaeta, Spirillum, and Spirodiscus.

BACTERIUM.

Character: Animal e familia Vibrioniorum, divisione spontanea in catenam filiformem rigidulam abiens.

Charactère: Animal de la famille des Vibrionides, prenant par la division spontanée la forme d'un fil articulé raide (p. 75).

Ehrenberg's Bacterium was a motile organism, and he also saw, or thought he saw, an organ of motion, and carefully figured it.

Ich habe auch bei der stärksten Art und Gattung *Bacterium* ein Bewegungsorgan als einfachen wirbelnden Rüssell erkant (p. 74).

Besonders erfreulich war mir der deutliche Wirbel am Vordertheil der kleinen Körper im farbigen Wasser, und eine angestrengte Untersuchung brachte mir sogar einen einfachen fadenartigen kurzen Rüssel zu directer Anschauung. Bei den grössten Formen [of *B. triloculare*] hatte der Rüssel 1/3 der Körperlänge, bei den kleinen die Hälfte. Die Bewegung der Thierchen war zitternd und um die Längsaxe wälzend (p. 76).

In this species he never saw more than 5 nor less than 2 or 3 septa. He never succeeded in making them take up stains, such as indigo, carmine, and india ink. "Vom Fortpflanzungsverhältniss sind nur Ei-? Körnchen und Selbsttheilung, ein rein thierischer Character, erkannt" (p. 74). At this time his genus was reduced to one species, that with which he started out, viz, *B. triloculare.*

> Ganz sicher ist nur eine Art der Gattung (p. 75).
>
> Von den im Jahre 1828 aufgeführten 3 Arten ist nur *B. triloculare* als Stamm verblieben, die beiden andern, *B. scintillans* und *simplex*, sind unter diesen Namen zur Gattung *Monas* gestellt (p. 77).

In the Vibrionides stress is laid on the incomplete self-division by which they form motile jointed threads (bewegte Gliederfaden) (p. 74). Bacterium is non-flexile (unbiegsam); Vibrio is flexile (schlangenförmig biegsam) (p. 75). Bacterium can only swim straight ahead. There is a more decided "Einschnürung und grösseren Isolirung der Einzelthiere" in Vibrio than in Bacterium. In both genera division takes place at a right angle to the long axis.

Bacterium triloculare (fig. 138) consisted of oval corpuscles developing into short cylinders with rounded ends, 2 to 5 times as long as broad (usually 3 times), having as many transverse rays [septa]. It was found in 1820 in swamp water in the oasis of Jupiter Ammon, in Libya. In 1831, near Berlin, Ehrenberg found a supposedly similar organism which he named *Bacterium articulatum.* Subsequently he says he rediscovered this organism in standing, "modrigen" water in a glass in his room, and finding what he considered to be transition forms, he abandoned the latter name and united all of these organisms, whatever they may have been, under the name of *B. triloculare.* The statements about the flagellum, and the figures of 1838, appear to have been drawn wholly from the Berlin forms, supposed to be identical with the African ones. The organisms were colorless and non-flexile and self-division was observed. Contents very finely granular. Sluggishly motile, but "zahlreich und deutlich durcheinander fahrend." The size of the African form is said to be $\frac{1}{300}$ linie; the Berlin forms were $\frac{1}{400}$ to $\frac{1}{192}$ linie. The single "Thierchen" in the Berlin form was one-fifth as long, *i. e.*, $\frac{1}{960}$ linie. Ehrenberg's figures represent a magnification of × 290 and × 1,000 and are much better than those of *B. triloculare* in the earlier work. They show a transversely 2 to 5 septate, rod-shaped organism, with rounded extremities, and bearing one polar flagellum about one-third the length of the body. There are no paired rods, or constrictions at any of the septa, but some of the rods are slightly curved. The shape and septation of the figures is slightly suggestive of some of the drawings of de Bary's *Bacillus megaterium* (Pilze Mycet. u. Bact., fig. 194). They also look somewhat like some of the involution forms of *Bacillus hortulanus* (Phil. Tr. Royal Soc., Series B, vol. 191, pl. 16). Both of these organisms, however, have peritrichiate flagella. The flagellum resembles that on species of Vibrio.

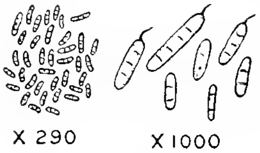

Fig. 138.*

Cohn's drawings of *Bacterium termo* are shown in fig. 139, copied from his "Beiträge" (Bd. I, Heft 2, Tafel III). Cohn did not consider motility of any generic value, and consequently paid no attention to organs of motion. Dallinger & Drys-

---

*Fig. 138.—Ehrenberg's *Bacterium triloculare*, showing flagella. From Die Infusionsthierchen, Plate V, fig. I, 1, 2.

dale's conception of this organism, at a time when the air was full of talk of Cohn's researches, is shown in fig. 140. Dallinger & Drysdale's drawings were made from unstained material, and there is no doubt that these expert microscopists actually saw what they figured, viz, a schizomycetous organism provided with one polar flagellum and belonging to the family Bacteriaceæ. Dallinger afterwards carefully measured the diameter of the flagellum many times over in unstained material, grown in Cohn's fluid.

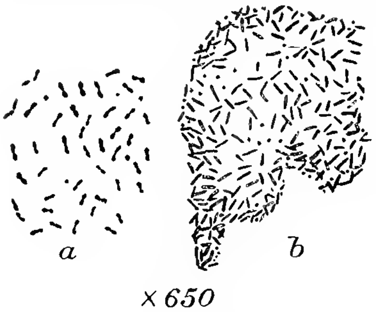

Fig. 139.*

As bearing on the question whether Ehrenberg could see the flagellum of an unstained bacterium with the microscopes at his disposal, it is interesting to note Dallinger's statement that Koch could not see the unstained flagellum of *Bacterium termo* because he used "low-angled glasses, which are incompetent to that demonstration." Another remark of Dallinger is also pertinent. "I have learned," he says, "from experience that there is as great a diversity in different individuals in the sensitiveness of the retina as there is in sensitiveness of the olfactory or auditory nerves."

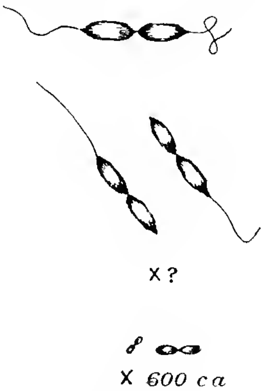

Fig. 140.†

The writer's own conception of *Bacterium termo* is shown in fig. 141. These organisms are green-fluorescent species cultivated in Cohn's solution, from water into which beans had been thrown in the manner described by Cohn. The very distinct flagella were stained by Löwit's method. The particular species from which this was obtained did not liquefy gelatin.

To the writer, then, the genus *Bacterium* is *Bacterium* (Cohn emend.), and is based on the morphology of the green-fluorescent organisms, capable of growing in Cohn's nutrient solution and called by him *Bacterium termo*.§ It corresponds to Migula's genus *Pseudomonas*, for which name it should be substituted as a proper generic name for straight or slightly curved Bacteriaceæ, motile by means of one to several polar flagella. It includes most of the yellow bacteria and all of the green-fluorescent bacteria (vide Migula's system, Bd. II, p. 875).

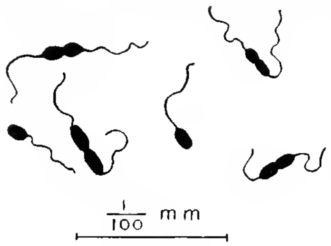

Fig. 141.‡

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*Fig. 139.—*Bacterium termo: a*, motile form; *b*, zoogleæ. After Cohn. Untersuchungen uber Bakterien. Beitrage z. Biol. d. Pflanzen, Bd. I, Heft 2, Plate III.

†Fig. 140.—Dallinger and Drysdale's conception of *Bacterium termo*. See Dallinger and Drysdale "On the Existence of Flagella in *B. termo*." Monthly Micros. Jour., Sept. 1, 1875, Plate CXIII, p. 105, figs. 6 and 7.

‡Fig. 141.—The writer's conception of Cohn's *Bacterium termo*. Organism obtained by throwing beans into water and then making a transfer from the green-fluorescent liquid to Cohn's solution. Stained by Löwit's method. × 2000.

§These organisms have no necessary connection with *Bacterium termo* Ehrenberg or with *Monas termo* Müller. We shall never know what these were.

We have therefore the following:

Bacterium (Cohn emend.).

Type The one-flagellate, green-fluorescent schizomycetes, capable of growing in Cohn's nutrient solution. To these should be added all the morphologically similar, non-fluorescent and yellow species.

Synonym *Pseudomonas* Migula.

Among others the following plant parasites belong here:

Bacterium campestre (Pammel),	*B. pruni* (Erw. Sm.),
B. hyacinthi Wakker,	*B. vascularum* (Cobb),
B. phaseoli (Erw. Sm.),	*B. juglandis* (Pierce),
B. Stewarti (Erw. Sm.),	*B. malvacearum* (Erw. Sm.).

These changes leave no generic name for the anthrax organism and other non-motile forms.

The writer would like to name the anthrax organism and related forms in honor of the distinguished man who first pointed out the generic significance of non-motility in this organism, but who unfortunately selected for it the preoccupied name of Bacteridium. There is, however, already a genus Davainea in helminthology, and it does not seem wise to make another, even in botany. Bacteria are now classed as plants, but we do not know what may finally be done with them. It remains, therefore, to adopt some old name, if an unobjectionable one can be found, and if not, to devise some entirely new name for the non-motile bacteria. There are several old names not now in use, *e. g.*, *Metalacter* and *Melanella*, but so far as I have been able to determine, none of them were given to organisms at all resembling the anthrax organism, and for one reason or another all must be rejected. I therefore propose the name *Aplanobacter* (from Greek words meaning *without motion* and *a rod*), and shall use it as the generic name for the anthrax organism called Bacteridium by Davaine, Bacillus by Cohn and Fischer, and Bacterium by Migula. Under Aplanobacter I include all non-motile forms morphologically similar to the anthrax organism (*Bacillus anthracis* Cohn), the latter, however, being taken as the type of the genus:

Aplanobacter nov. gen. nom.

An unattached, non-motile, rod-shaped organism, destitute of chlorophyll and multiplying by fission, sometimes forming threads of considerable length. The type of the genus, in the family Bacteriaceæ, is that organism causing anthrax and most commonly known in literature as *Bacillus anthracis* Cohn.

For the present non-sporiferous forms, resembling *Aplanobacter anthracis*, are also included under this genus, but if it shall be decided, later on, that the difference between sporiferous and non-sporiferous forms is of generic significance, then the latter may be excluded. This genus, as now understood, includes *Aplanobacter anthracis* (Cohn) and many other non-motile species called *Bacillus* in most books, but *Bacterium* by Migula. For a list of the species see *Bacterium* (p. 279) in Bd. II of Migula's "System." A few species there given are now known to be motile.

Forms related to *Bacillus tuberculosis* Koch and *Bacillus leprae* Hansen do not seem to belong with the anthrax organism, and some name must be found for these.

Lehmann & Neumann have suggested *Mycobacterium*, and we may use this name without in any way committing ourselves as to the significance of the branching forms. I would include also under it *Bacillus diphtheriæ* Loeffler (Corynebacterium L. & N.). The writer has not inquired critically as to whether this is the earliest available name for this group, but that of *Sclerothrix*, given by Metchnikoff in 1888, is twice preoccupied, and that of *Cocothrix*, given by Lutz in 1886, is too near the earlier Cocothrichium Link. In 1889, in Saccardo's Sylloge Fungorum, De-Toni and Trevisan included these organisms under the genus *Pacinia* Trevisan, but Trevisan's original draft of this genus included only vibrios, his type being the organism causing Asiatic cholera.

Another difficulty is to decide what name shall be used for the cause of Asiatic cholera and its relatives. The majority, perhaps, of pathologists and bacteriologists use the genus name *Vibrio*. They understand by it small spirally bent organisms common in water and possessed of one polar flagellum or rarely of several such organs, the *Vibrio choleræ* being taken as the type. Others call most of these organisms Vibrio, but speak of *Spirillum choleræ*. Others use the two names Vibrio and Spirillum interchangeably. Others try to escape the difficulty by avoiding Latin names altogether, speaking in the same article indifferently of "the cholera vibrio," "the cholera bacterium," and "the cholera bacillus." This is the case frequently in the recent big monograph by Kolle & Wassermann. A few persons, following Migula, have used Schroeter's name, *Microspira*, given in 1886. Microspira is inadmissible, according to strict rules of priority, because Trevisan's name *Pacinia* is one year earlier (1885). Trevisan's genus, although badly defined, following Zopf's ideas of pleomorphism, is tied hard and fast to the cholera organism. Apparently this name was given without any personal acquaintance with the organism named, but according to current rules of nomenclature this makes no difference. The choice, therefore, is between Pacinia and Vibrio, the one tied fast to a known species, but not used by working pathologists or bacteriologists since it was coined, so far as my reading goes, the other in common use, but a floating name—that is, one which can not be used for bacteria, and at the same time tied to any definite species or group of species included in the original draft of the genus.

Müller's genus Vibrio was published in 1773 in his "Vermium terrestrium et fluviatilium." It contained 15 species—bacteria, eel-worms, etc. Other things were also afterward put into it by Müller, *e. g.*, diatoms. We will be content with the first draft of the genus. It is described as follows: "Vibrio. Most simple, inconspicuous, terete, elongate worms." The first species is described as follows:

Vibrio lineola.

Vibrio linear, hard to see. Danish, Stræg-strækkeren. A most minute animal, almost exceeding in smallness *Monas termo* and 30 times less than *Vibrio bacillus* and entirely different. A trembling motion of myriads of oblong and obscure points is seen in a single drop, or with the highest magnification undulatory movements. In infusions of vegetables it almost fills the substance of the water after many days.

The second species, *V. bacillus*, first obtained from hay infusions, is described at a little greater length, but not any better. The third species is a mixture of nematodes. The first two species are bacteria. One other species of schizomycete is described, viz, *Vibrio undula*. This last, or what was supposed to be it, together with *Vibrio spirillum*, a subsequent addition by Müller, was removed by Ehrenberg to form his genus Spirillum, which we still retain. The eel-worms were removed to form the genus Anguillula, and the other infusoria were variously distributed. Only the first two species of the original genus remained in Cohn's time, and neither one was used by him. Cohn used *Vibrio rugula*, one of Müller's additions, for the first species under his emended genus Vibrio, but this has now been put by Migula into Spirillum. The only other member of Cohn's genus Vibrio (emend.), *V. serpens*, is still less like the cholera organism. Ehrenberg's figure of *Vibrio lineola* Müller (Infusionsth.) shows crooked little organisms not unlike what we now call vibrios.

As a general proposition the writer believes that if a genus name is to be retained one should be able to tie it to some definite type-species, and it ought to be a species put into a genus when it was first published, and not one put in after the genus has been emended out of all recognition. Of course, nothing can be done with Müller's, or Cohn's, genus description of Vibrio. If the name is to be retained for any organisms whatsoever, the description must be made over and the name anchored to a known species. Ordinarily such a name should be discarded. Under the circumstances, we may perhaps strain a point, make over the genus description in toto, and use the name Vibrio, as many pathologists have done, for Koch's comma bacillus and related forms. Logically, perhaps, we should adopt the strange Pacinia; for convenience sake we may continue to use the familiar Vibrio. The name Vibrio is not used by helminthologists or algologists, and, if we connect it to the first species described by Müller under the genus, we may anchor the name to any small motile species, without fear that subsequent researches will require changes to be made. This may be done, because the description of Müller's *Vibrio lineola*, the first species, is so imperfect that identification is out of question; the name can never be attached to any morphologically definite organism or group of organisms different from the cholera vibrio, even the gelatinization of the water after many days being probably enough due to other bacteria. The writer follows Lafar (1st ed.), Alfred Fischer, Lehmann & Neumann, *et al.*, and would write:

Vibrio (Müller, Cohn, emend.).*

Type of the genus, Koch's comma bacillus.

Synonyms.—Spirillum choleræ-asiaticæ Koch; *Microspira comma* Schroeter; *Pacinia choleræ-asiaticæ* Trevisan.

Kendall has criticized Migula's use of the word Pseudomonas on the ground that he has combined under it two distinct groups of the family Bacteriaceæ, the monotrichiate and the lophotrichiate forms, and because the name implies, he says, a relation to "pseudomonads." The second criticism implies that to be tenable a name must conform etymologically to all the facts in the case. This is a misconception. No one is warranted in setting aside a generic or specific name simply because it seems inappropriate. It is not inappropriate, however, since the first species in Müller's genus *Monas* was undoubtedly founded on small bacteria of some sort. As to the first criticism, that lies also against my use of Bacterium and requires a word. This criticism appears to me not well taken, since in the Bacteriaceæ, as Migula first pointed out, there is no such sharp distinction between the monotrichiate and the lophotrichiate forms as there seems to be among the Spirillaceæ. Within the limits of the same species, and on the same cover-slip, forms may occur with one flagellum and others with two or more (see fig. 15, which is not the only one I might offer). The name Pseudomonas is of earlier date than Fischer's or Kendall's equivalents, has priority, and can not be set aside on the grounds named. If the reader is not satisfied with the reasons I have given for substituting the earlier name *Bacterium*, then he should continue to use the name *Pseudomonas.*

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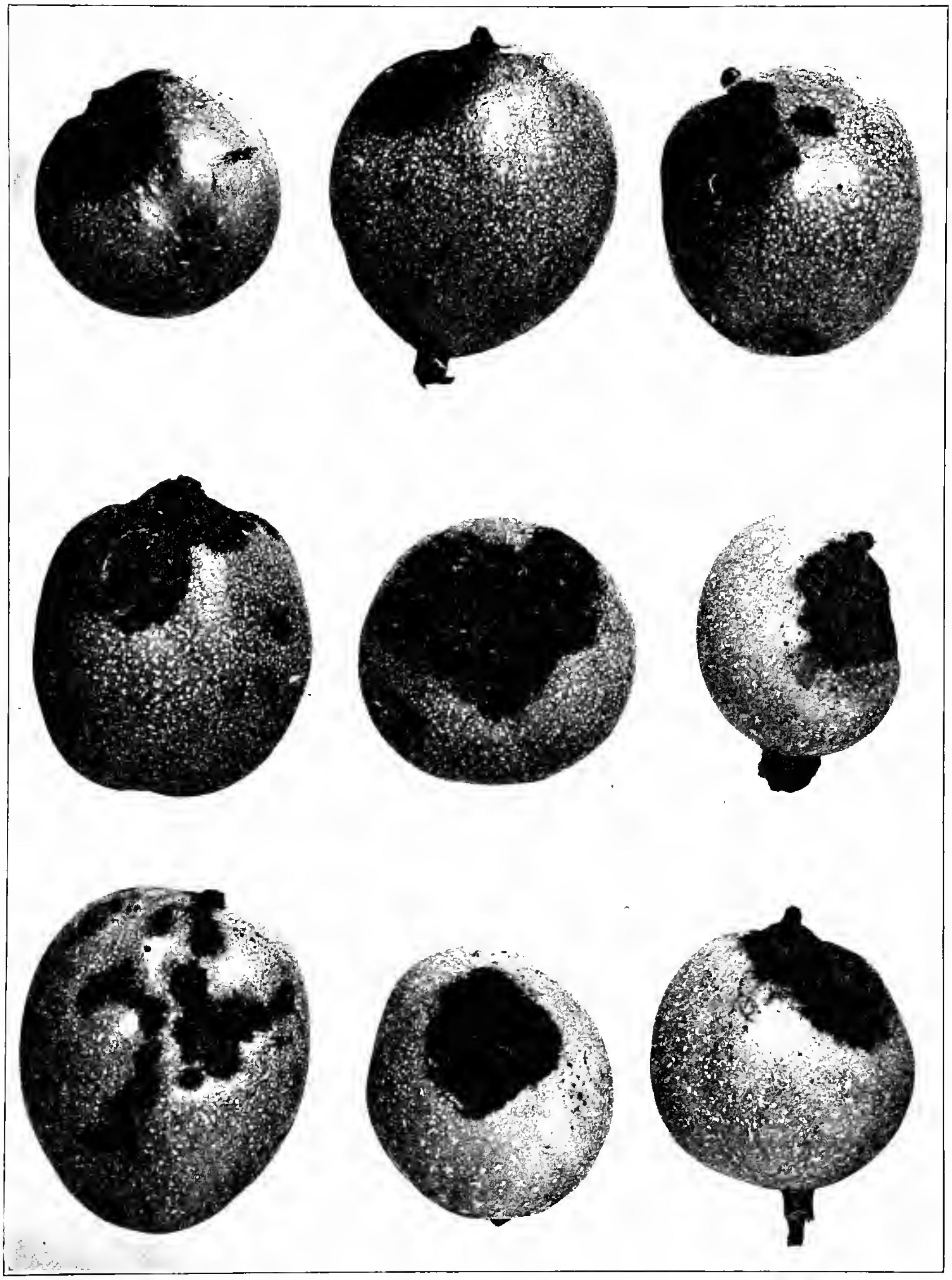
*According to Fischer, 1903, and Lehmann & Neumann, 1896, this emendation was made by Loeffler.

For the present, therefore, I follow Migula's classification, except in so far as relates to his use of the words Microspira, Pseudomonas, and Bacterium.

The following names should be rejected:

Acromatium.
Actinobacter.
Actinomyces.
Aërobacter.
Aethylbacillus.
Amylobacter.
Arthrobacter.
Arthrobactridium.
Arthrobactrillum.
Arthrobactrium.
Ascobacillus.
Ascobacterium.
Ascococcus.
Astasia.
Astrobacter.
Azotobacter.
Babesia.
Bacteridium.
Bacteriopsis.
Bactrillum.
Bactrinium.
Bolliugera.
Botryomyces.
Cenomesia.
Chromatium.
Clathrocystis.
Clostridium.
Clostrillum.
Clostrinium.
Coccos.
Cocobacillus.
Cocobacteria.
Cocothrix.
Cohnia.
Cornilia.
Corynebacterium.
Cryptococcus.
Cystobacter.
Dicoccia.
Diplectridium.
Diplobacteria.
Diplococcus.
Discomyces.
Dispora.
Erebonema.
Erythrobacillus.
Erythroconis.
Fenobacter.
Gaffkya.
Gallionella.
Gliabacteria.
Gliacoccus.
Glischrobacterium.
Gonococcus.
Granulobacter.
Gummibacillus.
Hæmatococcus.
Halibacterium.
Helicomonas.
Helobacteria.
Hyalococcus.
Iodococcus.
Klebsiella.
Kurthia.
Lactobacter.
Lampropedia.
Leptothrix.
Leucocystis.
Leuconostoc.
Lineola.
Macrococcus.
Megabacterium.
Megacoccus.
Melanella.
Meningococcus.
Merismopedia.
Mesobacterium.
Mesococcus.
Metallacter.
Microbacterium.
Microhaloa.
Microphyta.
Microsphæra.
Microspora.
Microsporon.
Microzoa.
Microzyma.
Monas.
Monobacteria.
Monococcus.
Mycoderma.
Myconostoc.
Mycothece.
Mycothrix.
Neisseria.
Newskia.
Nitrobacter.
Nitrosococcus.
Nitrosomonas.
Nocardia.
Nosema.
Octopsis.
Ophidomonas.
Pacinia.
Paracloster.
Paraplectrum.
Pasteurella.
Pasteuria.
Pediococcus.
Perroncitoa.
Petalococcus.
Photobacillus.
Photobacterium.
Photospirillum.
Plectridium.
Pleurococcus.
Pneumobacillus.
Pneumococcus.
Pollendra.
Proteobacter.
Proteus.
Rhabdomonas.
Rhizobium.
Saccharobacillus.
Saccharobacter.
Schinzia.
Schuetzia.
Sclerothrix.
Sphærococcus.
Sphærotilus.
Spirobacillus.
Spirodiscus.
Spiromonas.
Spirulina.
Sporonema.
Streblothrichia.
Streptobacillus.
Streptobacteria.
Streptothrix.
Tetracoccus.
Thermoactinomyces.
Thermobacillus.
Thermobacterium.
Thioderma.
Thiosphæra.
Thiosphærion.
Torula.
Tyrothrix.
Ulvina.
Urobacillus.
Urobacter.
Urocephalum.
Urococcus.
Urosarcina.
Zooglœa.
Zopfiella.



Walnut disease.

Bacterial black spot of the Persian walnut (Juglans regia), more commonly known as the English walnut. Half-developed green fruits from an orchard in California, showing the badly spotted epicarp; spots due to Bacterium juglandis (Pierce). Leaves and shoots are also subject to this disease, which has become serious in Southern California, where large quantities of these nuts are grown for market. The attacked parts are conspicuously blackened as if charred. The numerous small white spots show the location of groups of stomata. Infection takes place readily through unbroken tissues.

A very few of the preceding may perhaps some time make good their claim to be considered as independent genera. Many of these names are preoccupied in this group or in other groups; some represent mixtures; others, purely physiological genera; but some of them may be used within the limits of genera to designate special physiological groups whenever such use leads to clearness of understanding.

Naegeli, Beyerinck, and Winogradsky have studied especially the food requirements of bacteria. Many others have, of course, contributed. Alfred Fischer has given a good summary in the second edition (p. 96) of his "Vorlesungen." Following this, and considering them especially with reference to their nitrogen-nutrition, the bacteria may be classified into seven groups:

1. Paratrophic bacteria. The obligate parasites, capable of growing only on substrata similar in composition to the fluids of the host.
2. Peptone-bacteria. Organisms requiring peptones or albumoses.
3. Amido-bacteria. Organisms which also grow well when their nitrogen food is restricted to amido-bodies—asparagin, leucin, etc.—but not able to use ammonia.
4. Ammonia-bacteria. Able to take nitrogen from ammonia compounds.
5. Nitrobacteria. The denitrifying organisms. They require organic carbon compounds.
6. Nitrous and nitrate bacteria. The saltpeter-bacteria. Nitrates, nitrites, or ammonia-compounds furnish the necessary nitrogen. The carbon dioxide of the air serves as their carbon-food.
7. Nitrogen-bacteria. Organisms able to assimilate free nitrogen, but only in the presence of organic carbon compounds.

In 1895 Wyatt Johnston suggested that all the important characteristics of a species might be recorded by numbers arranged in a definite order. Gage & Phelps and Kendall afterward made use of the Dewey numeral system. By this means the leading features of a hundred or of five hundred organisms might be recorded on a single page, so as to be very easily compared. Chester has modified this system for application within the genus as follows:

100.	Endospores produced.
200.	Endospores not produced.
10.	Aerobic and facultative anaerobic.
20.	Anaerobic.
1.	Gelatin liquefied.
2.	Gelatin not liquefied.
0.1	Acid and gas from dextrose.
.2	Acid without gas from dextrose.
.3	No acid from dextrose.
.01	Acid and gas from lactose.
.02	Acid without gas from lactose.
.03	No acid from lactose.
.001	Acid and gas from saccharose.
0.002	Acid without gas from saccharose.
.003	No acid from saccharose.
.0001	Nitrates reduced.
.0002	Nitrates not reduced.
.00001	Fluorescent.
.00002	Violet chromogens.
.00003	Blue chromogens.
.00004	Green chromogens.
.00005	Yellow chromogens.
.00006	Orange chromogens.
.00007	Red chromogens.
.00008	Brown chromogens.
.00000	Non-chromogenic.

According to this scheme the formula for *Bacillus coli* and *Bacterium campestre* would be respectively B. 212.11110 and Bact. 211.33315. Such a system admits of indefinite extension, and the reader can see at a glance that, if well worked out so as to include all the more important facts, it would be invaluable for unification of methods and for quick, easy reference. Each group of digits should include as many facts as possible. Kendall, for instance, under the gelatin group has also included action on dextrose as follows :

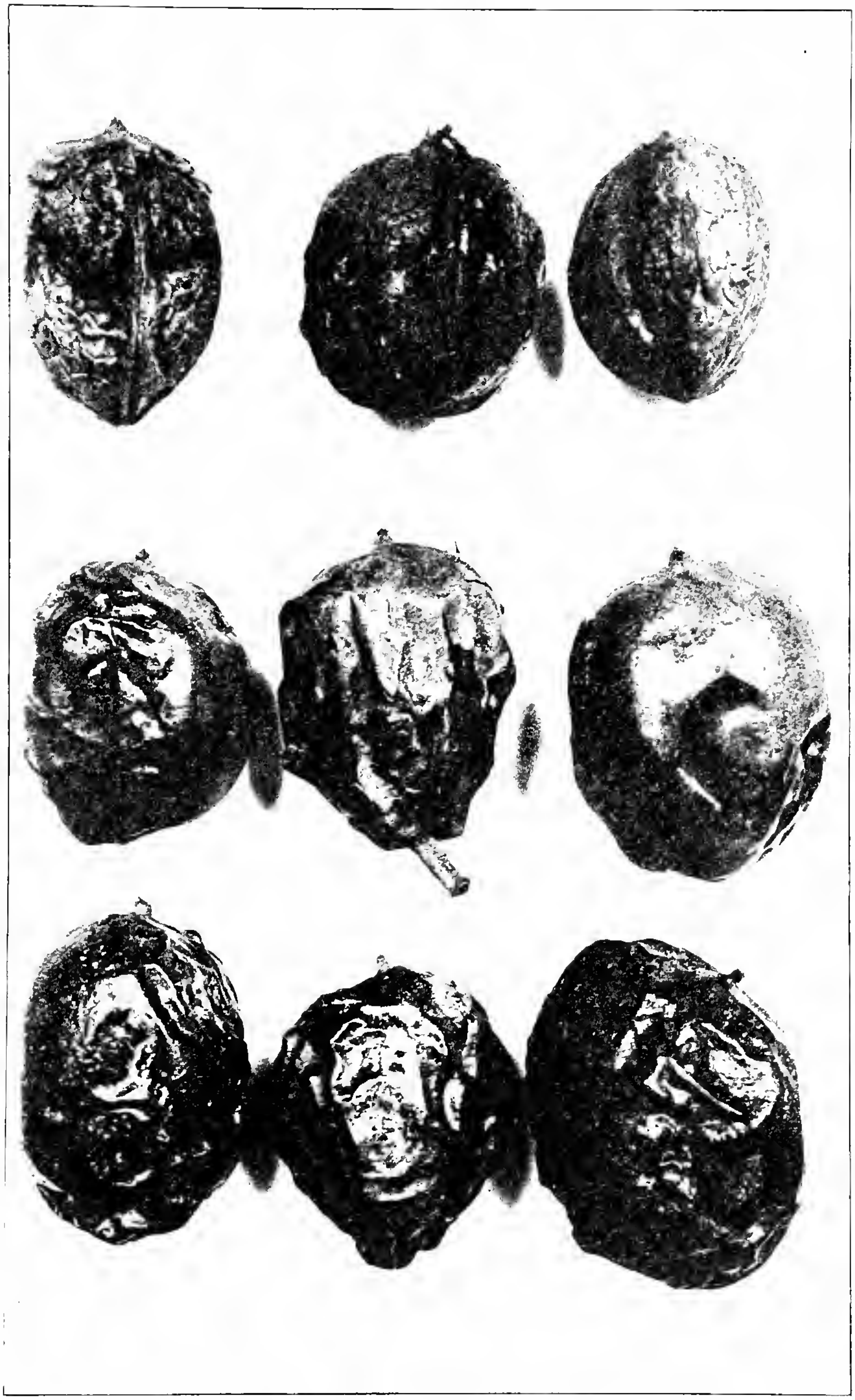
	Liquefaction of gelatin.	Fermentation of dextrose, gas production.	Acid production.
1.	Negative.	Negative.	Negative.
2.	Negative.	Positive.	Positive.
3.	Negative.	Negative.	Positive.
4.	Positive.	Negative.	Negative.
5.	Positive.	Positive.	Positive.
6.	Positive.	Negative.	Positive.
7.	Unknown.	Negative.	Negative.
8.	Unknown.	Positive.	Positive.
9.	Unknown.	Negative.	Positive.

The subject is now in the hands of a committee of the Society of American Bacteriologists for consideration and recommendation, and criticisms are desired. They may be sent to Prof. F. D. Chester, Wilmington, Del.; Prof. F. P. Gorham, Providence, R. I., or to Erwin F. Smith, Washington, D. C.

VALUE OF MORPHOLOGICAL CHARACTERS.

Ebb and flow, growth and change, this is the order of the world. Living things conform to a certain set of conditions and we say they are constant in structure and function because the conditions are fairly constant; change the environment too much and they are destroyed; change it essentially, ever so little, and the animal or plant begins at once to respond to it. This is especially true of simple unicellular forms. We can not, then, expect more than a moderate amount of constancy in these low forms of life. If under slight changes of environment they are fairly constant morphologically, it is all that we can expect, and in interpreting all descriptions we must make due allowance for these slight changes which an author may not have observed.

There have been two extreme views respecting the morphology of the bacteria. Béchamp, Hallier, Billroth, and Zopf stand for one extreme; Koch's earlier views for the other. To Hallier bacteria were only the developed plastids (protoplasmic granules) of fungi, and under widely different forms we might have the same organism functioning at one time as a harmless mold and at another as a micrococcus, causing the dreaded cholera or some other human or animal disease. Béchamp's microzymas were granules or fundamental elements more minute than the plant or animal cell, granules out of which all life developed and which persisted in other forms after the death of the cells. To Billroth all ordinary forms of bacteria, however dissimilar they might appear, were but stages of one unique species, viz, his *Coccobacteria septica*. Zopf did not carry his doctrine so far, but taught pleomorphism as a fundamental characteristic of the bacteria. To-day an organism might be a Micrococcus, tomorrow a Bacterium or a Bacillus. Koch, on the other hand, insisted on the fixity of forms. To him a bacillus was always the same thing, and the views of the polymorphists were explained as the result of errors in technique, the confounding of entirely different things. Koch's own methods were exact and his views had



Bacterial black spot of the walnut.

A late stage of the disease on the nuts. Photograph by Pierce. Mr. Pierce, who discovered the cause of this disease, has demonstrated 50 per cent of the losses preventable by spraying, and is now endeavoring to obtain resistant varieties by hybridizing and selection. The sum of $20,000 was offered by the walnut growers of California some time ago for a satisfactory remedy, and recently the legislature of California has appropriated a considerable sum for its investigation.

enormous weight, since he depended not on mere assertion, but pointed out many errors of fact and many flaws in the reasoning of his antagonists.

To-day the majority of bacteriologists hold a sort of middle ground. Very few are willing to accept the views of the old polymorphists, but there is a spirit of rational inquiry abroad. We know that bacteria are much more responsive to changed environment than was supposed by Koch and his followers in the eighties, and we are prepared to believe anything respecting their origin and their polymorphism which can gain the suffrage of the great body of critical workers who now cultivate this field, and who at once begin to investigate from all sides any new and strange statement. Duplication of work, so called, is not waste of time.* If sharp criticism abound, so much the better. In this way we shall gradually reach a clearer understanding of these organisms. Meanwhile, let each one cultivate his own little field as best he may, and, above all, let him be very sure of his facts before he publishes.

There can be no doubt that the same organism sometimes exists as a long filament in which no septa are visible and at other times as a short or nearly isodiametric rod, but we are not thereby compelled to consider the short form as a Micrococcus, *i. e.*, as something very different from the long form. Physical conditions probably have much to do with bringing about these differences. Respecting the meaning of the branched forms, described by so many writers, the author is in doubt and can only wait for more light. Several hypotheses are open: (1) The bacteria, as now understood, are not a homogeneous group, but consist of many organisms of dissimilar origin and differing morphologically, which will be gradually separated out and put into their proper places, just as the Oosporas (Streptothrices) have already been removed, leaving as *Eu*-bacteria a genuine residuum of morphologically similar forms; (2) the bacteria do not any of them represent a natural group, but are stages of various higher forms, just as certain cells, multiplying indefinitely in yeast form, are now known to be conidial stages of the higher fungi (smuts, mucors); (3) the branched forms, which come mostly in old cultures, or in other crowded conditions where the organisms are subject to the injurious action of their own by-products (root-tubercles of Leguminosæ, lung-tubercles, etc.), are to be regarded simply as involution or degeneration forms, and not higher stages of development; (4) the branchings are incomplete longitudinal fissions favored by special chemical or physical conditions. Time will show where the truth lies.

No harm will come to any one if all of these perplexing questions are not settled definitely within his own generation.

So far as can be judged from structure the bacteria appeared in early geologic ages (in coprolites, decaying bones, tree-trunks, etc.) in forms closely resembling those now existing, but we have very little definite information as to their origin. Probably they are related to the lower algæ and of as ancient origin. On relationship of the bacteria to Algæ, Fungi, Flagellata, and Myxomycetes, see Migula's remarks on the systematic position of the bacteria, in his "System," part I, page 237.

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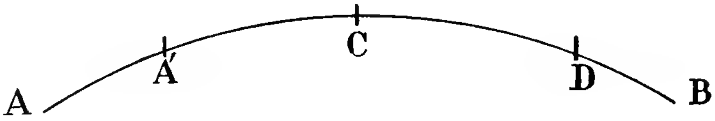
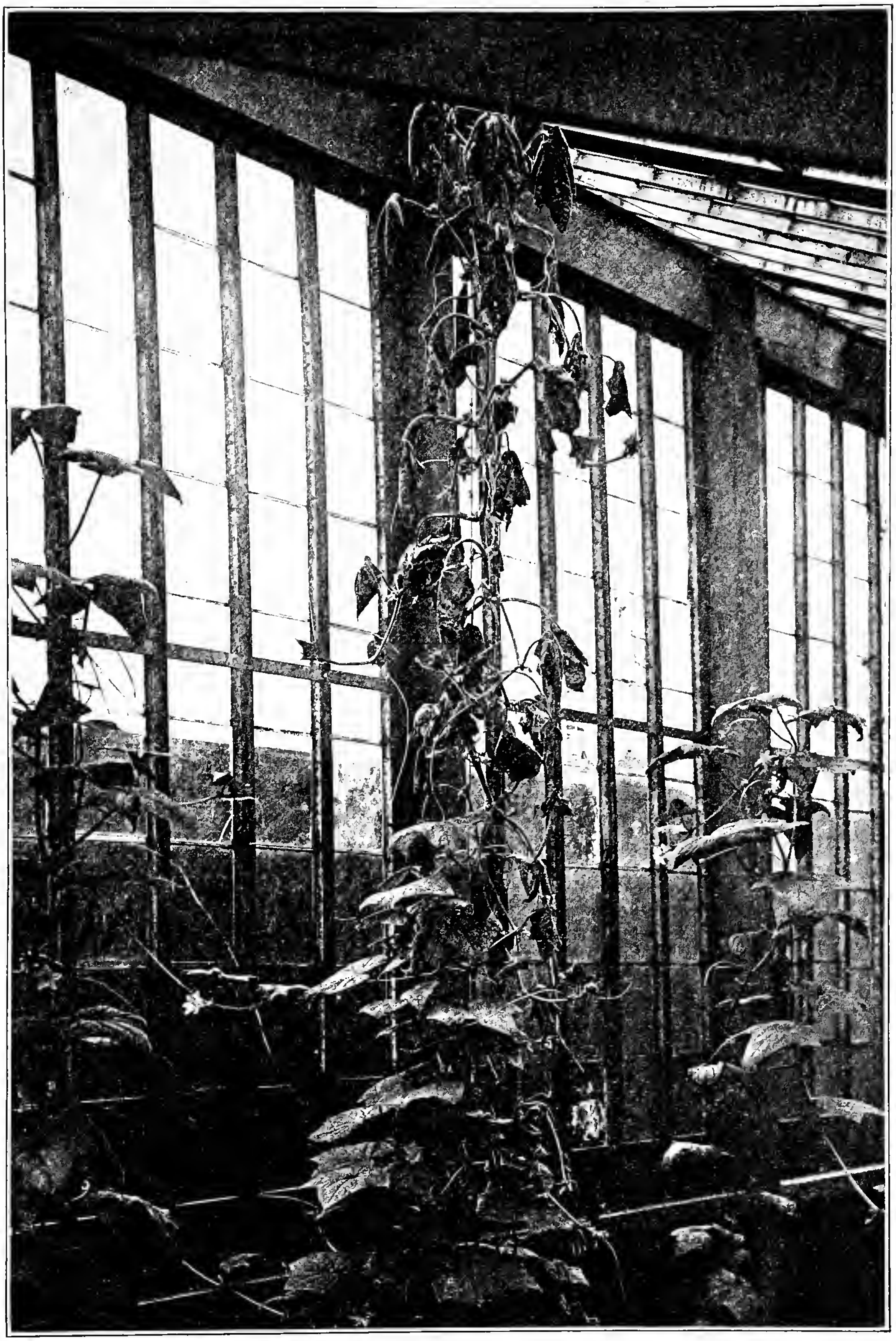
*Karl Pearson has recently stated that 50 per cent of the scientific work of the 19th century will have to be junked as worthless. In bacteriology 75 per cent would be nearer the truth.

VALUE OF CULTURAL CHARACTERS.

Of what worth are the cultural characters commonly mentioned in descriptive bacteriology? Much depends on the proper answer to this question. There are undoubtedly two extreme views, neither of which is correct. One investigator would maintain that no dependence can be placed on them; another seems to have no suspicion of any source of uncertainty. The truth undoubtedly lies somewhere between the two. That great progress in bacteriology has come from their use must be admitted by all. To cast doubt on everything already done is only to bring chaos back again. It is wise to make haste slowly. No necessity exists for making a rubbish heap of the past before beginning one's own work. Old methods should be tried repeatedly, scrutinized from every standpoint, and only abandoned when they have yielded all that can be obtained from them, or when there is something distinctly better to take their place. New methods should be hailed with enthusiasm only in so far as they have actually made good their claim to be genuine improvements. A great deal of writing on bacteriology is worthless because not based on well-considered and properly conducted experiments. Hypotheses ad libitum, the more the better; but let us not forget to test each one in the crucible of experiment, and generally *before publishing*, *rather than after*. In other words, give to the world only the well-established facts. As a means toward arriving at the truth, let each person not only experiment as carefully as possible, but let him set down all the steps in his procedure, so that others may repeat his experiments. Many misapprehensions and supposed contradictions arise from the fact that workers are led to believe they have exactly duplicated another man's work when they have done nothing of the kind. The temperature at which they have worked has been different, or some other physical or chemical condition, important but not recognized or not recorded by the first writer, has been unlike, and the results are not the same. Bacteria are not so simple as they appear. While monotonous morphologically they are complex in their multitudinous physiological activities, and are extremely apt to vary under a slightly changed environment. When we repeat an experiment we must know, therefore, whether we have preserved substantially the former environment. If we have not, then it should not surprise us if the results are somewhat different from those we anticipated.



A very frequent source of error in interpreting descriptions consists in not making sufficient allowance for changes due to slight variations in the culture-media. I can perhaps make my meaning plainer in the following way: Let the curve A B represent all the variations in color and appearance of a given organism on a given medium, *e. g.*, steamed potato. Now, if a worker describes his organism from a



Bacterial wilt of the cucumber.

(Introduced to illustrate transmission of the disease by insects). The central plant (variety Long green) was inoculated on June 17 with Bacillus tracheiphilus by the striped cucumber-beetle (Diabrotica vittata). As a result the gnawed leaves first wilted and then the whole upper part of the plant, the vascular bundles being occluded by the sticky white slime of this bacillus. Photographed July 1, 1905. About 1-14 natura size. The entire plant was dead about two weeks later.

few cultures, he will then in all probability have covered only a fraction of the curve A B, let us say between C and D, and not the whole curve of growth. If, now, another worker should happen to experiment with potatoes capable of giving rise in the organism to phenomena represented by that part of the curve lying between A and A′, he would get somewhat different results and yet this would not prove steamed potato to be a worthless culture-medium. The only real facts in the supposed case are that neither person has experimented sufficiently to draw up a proper description of the characteristics of the given organism on potato. Let us suppose we have to do with a yellow organism, *e. g.*, *Bacterium phaseoli* and that A to A′ represents a pale yellow growth, with no graying of the potato, while D to B represents a very deep yellow growth, with very decided graying of the potato. The cultures look like different organisms, but they are not. The descriptions would differ. Neither account alone would form a proper description of the behavior of this organism on potato, but there should be rather a combination of the two and of all intermediate stages, viz—Potato : Color varying from pale to deep yellow, flesh of the potato usually grayed, but sometimes remaining unchanged, etc. The same remarks apply to other non-synthetic media.

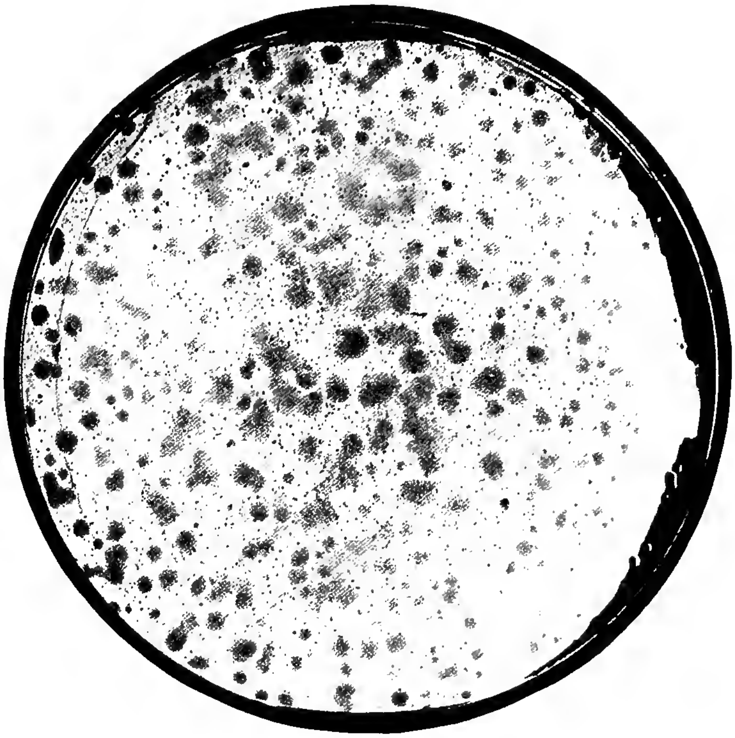


Fig. 142.*

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*FIG. 142.—Iris-rhizome rot. A dense sowing of the organism in an agar-plate culture after 45 hours at 25° C. The buried colonies small. Not van Hall's organism, which, as received from Král of Prague, is non-pathogenic in my hands.

In case of agar and gelatin there are numerous variations due to inadvertent changes in the culture-medium, especially if this is made by students. The media should be made by competent, experienced persons, and then the descriptions of the behavior of the organism on it should be broad enough to include slight differences in the aspect of the colonies, streaks, and stabs, which often depend on chemical and physical conditions within the control of the experimenter, *e. g.*, on the water-content, on age of the medium, amount of moisture in surface-layers, kind of peptone, kind of gelatin, length of exposure and degree of heat during sterilization, etc. The dense or thin sowing of the plate may sometimes make a very decided difference in the aspect of the colonies. Fig. 142 shows a densely-sown plate, the colonies round or roundish. Fig. 143 shows the same organism, and from the same set of plates, but thinly sown and two days older. Here the colonies are radiate. In case of *Bacillus aroideæ* when grown on agar-plates, near the maximum and minimum temperature limits, the surface-colonies are round even after many days, but they are promptly and strongly radiate when grown at or near the optimum temperature (see figs. 144, 145). When very thin sowings of this organism were exposed to the high temperature, the colonies were also round. It occurred to the writer that the round colonies obtained on the agar-plates exposed in the thermostat at 37° C. might be due to physical changes in the surface

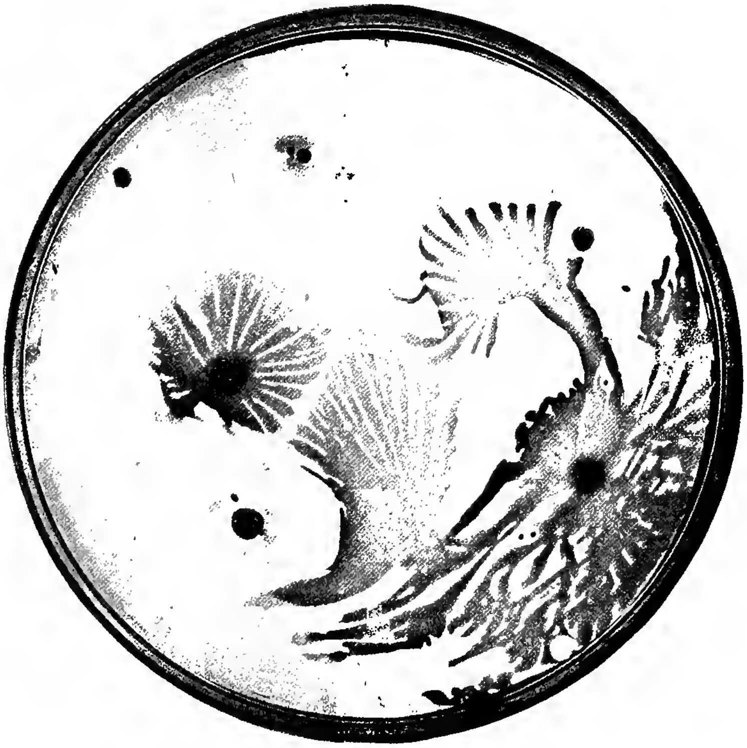

Fig. 143.*

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*Fig. 143.—Iris-rhizome rot. The same as 142, but sown thinly and kept for 4 days at 25° C.

layers of the agar, *i. e.*, to rapid loss of water, and experiments have shown this to be the case. Two sets of Petri-dish poured plates were made, inoculating from the same culture. One set was exposed in the open thermostat at 37° C., and these developed round colonies, similar to those shown in fig. 144. The other set was inclosed in the same thermostat, but inside of a closed glass vessel containing water. The colonies on these grew in radiating form, the same as in a third set of plates exposed at 30° C. This does not account, however, for the appearance of circular colonies at low temperatures. After twelve days' exposure in an ice-box the writer obtained the same result as Townsend; the colonies were not radiate, but looked like those shown in fig. 144.

UNDERGRADUATE WORK.

As a rule, the results of this kind of investigation are to be distrusted. The fresh ambition of students and their delightful eagerness to take up hard problems are sources of great pleasure to every good teacher. At the same time such students must be held back rather than urged on, since for the most part they are still unfitted to do independent work, especially that which involves the drawing of general conclusions from a variety of experiments. The ordinary training of botanical and zoological laboratories will not fit the student for specialization in pathology and bacteriology. Skill in this sort of work must be obtained from consorting with the professional pathologist and bacteriologist. In general, at the present time a well-equipped modern laboratory devoted to animal pathology is a much better place for the plant bacteriologist to learn methods than even our best-equipped botanical laboratories. One of two alternatives is open to the ambitious student. Either he must submit to a long and rigorous course of elementary study in a bacteriological laboratory, under a competent *and critical* teacher, or else he must be content to pick up the general principles of the science out of books and journals, with much blundering and stumbling in the first years of his study. During this nursery period, if he is jealous of his own reputation, he will not publish much. My experience has led me to discount very liberally the conclusions of student investigators, and I consider those students very unfortunate whose teachers urge them into precocious publication. In many cases nothing could be more damaging to their own reputation as scientific inquirers, or more injurious to the progress of science. Bad papers also react upon the teachers of such students, who can not by any shift evade responsibility. My advice to teachers is to discourage all students who do not show marked aptitude, and to give to those who do show signal ability the best possible training in *methods of work*, but to discourage them from undertaking difficult pieces of original investigation. The only alternative is for the teacher to follow their work step by step and assume joint responsibility for it in the end. Even this latter course is sometimes risky, as the history of science shows very conclusively.

After a year or two of careful work on methods, under the watchful supervision of a good teacher, the bright student will have learned how to avoid many of the pitfalls which beset his way, and, if he has acquired a proper training in other directions, such as general botany, modern physics, chemistry, the modern languages, etc., he may be trusted to undertake some original research. Even when once on his feet as an investigator, my advice to him would be: Try every conclusion repeatedly and make haste slowly. When he becomes uneasy at delays, let him reflect that one really good paper does much to set an unknown worker on his feet among scientific men, whereas one or two hastily written, poor papers will injure his reputation as an investigator more than half a dozen good papers subsequently published will suffice to repair. Moreover, in this age of enormously multiplied publication it is impossible to read everything, and consequently if a writer wishes to attract attention he must have a commanding grasp of his subject; must present its leading features in a clear, interesting style; must be as brief as the importance of his subject will warrant, otherwise his words are certain to be overwhelmed and lost; and, finally, must publish in a proper place, *i. e.*, not in some obscure "Transactions" or in some local journal with a small circulation. *When ready to publish, stop and do your work all over again with more care.* This is my advice to beginners. In the course of such general revision the chances are that many statements will require correction or modification, and some may have to be omitted altogether.

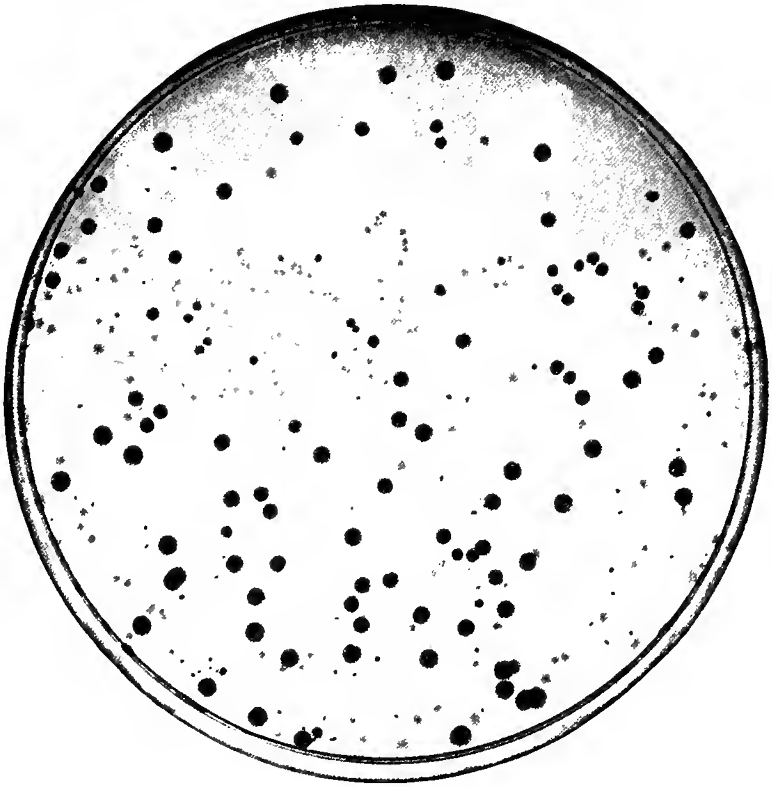

Fig. 144.*

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*Fig. 144.—Colonies of *Bacillus aroideae*, circular when grown on an agar plate at 37° to 38° C., *i. e.*, at a temperature near the maximum. Photograph by Townsend.

In any event, the student must have a considerable body of knowledge, *gained by actual experiment*, before his judgment is worth much. In the beginning he is apt to depend too much on the constancy of organisms and is certain to be misled by names. To illustrate: To him all agar is agar and all gelatin is gelatin. Not so, perhaps, to the organism with which he is experimenting. Slight differences in the composition of a culture-medium sometimes make considerable difference in the growth and general appearance of the bacteria, and this must be taken into account. After the student has passed this stage of development he can interpret his results much better. If, then, on some culture-medium he obtains results slightly different from those already published by some author, he is not immediately driven to suppose (1) that he has a new species, or (2) that the earlier writer was in manifest error. Other hypotheses now lie open to him. He is dealing with a living and variable organism, and perhaps the conditions in his experiment are not precisely like those to which it was subjected by the previous experimenter. It may also be an organism which has already varied into many races having slightly different peculiarities. Only when full weight has been given to these possibilities is he entitled to fall back on the others. On the other hand, however, he must not

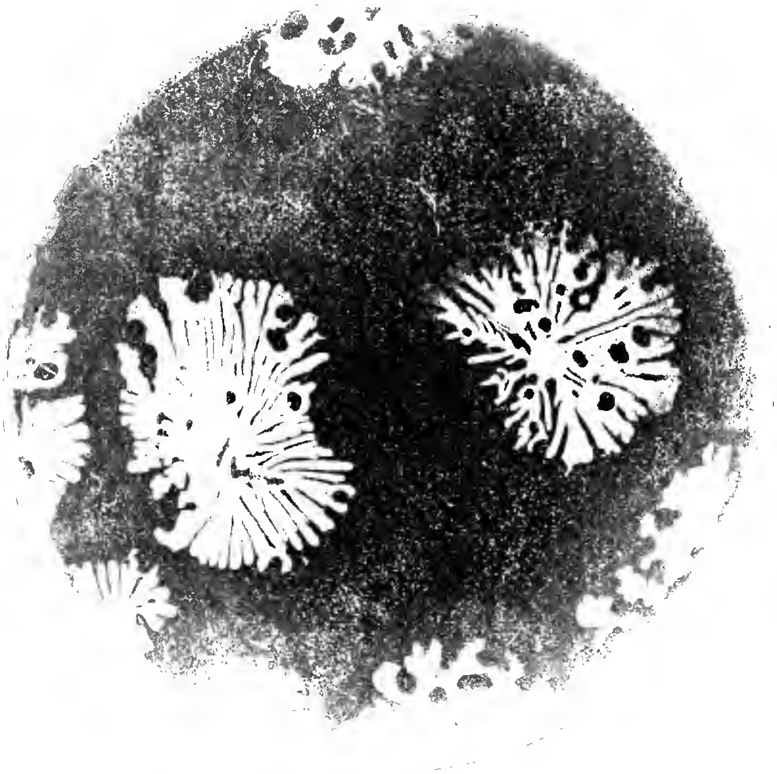

Fig. 145.*

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*Fig. 145.—Colonies of *Bacillus aroideae,* radiate-fimbriate when grown on an agar plate at 25° C. Photograph by Townsend.

escape Scylla only to fall into Charybdis. It may be that his organism varies in all sorts of ways, but he is by no means to assume this. Every hypothesis must be tried in the reducing fire of exact experiment.

Probably the best acquirement a student can get from his years of training is a spirit of self-distrust leading to habitual caution in the drawing of conclusions and the making of general statements. Such a spirit will preserve him from many foolish statements and will enable him to serve his generation to the best of his ability. He will not go far, however, without a tremendous earnestness, an indomitable energy, directed in proper channels. Let him concentrate this energy, the most priceless of all human attributes, and attack specific problems, one after another or a few at a time; not all at once. Honesty, industry, and self-reliance, tempered with the self-distrust already mentioned, will then carry him very far on the road he desires to go. Finally, the student should remember that the ideal man of science, and to a large extent also the actual man of science, is a modest man, always inclined to be cautious, always willing to revise his conclusions in the light of fresh evidence, generally plain-spoken, always an enemy of shams, and never offended by frank and honest criticism, preferring the white light of truth to the plaudits of the multitude.

A FINAL CAUTION.

Probably more mistakes arise from failure to carefully check up the work behind one than from any other source. What is meant by this can be explained in a few words, by means of a series of examples.

(1) I make subcultures from a poured-plate colony. The first subculture is on slant agar, the second is from the agar into beef-broth, the third is from the beef-broth into potato-broth, and from the latter I propose to inoculate a plant. The in-

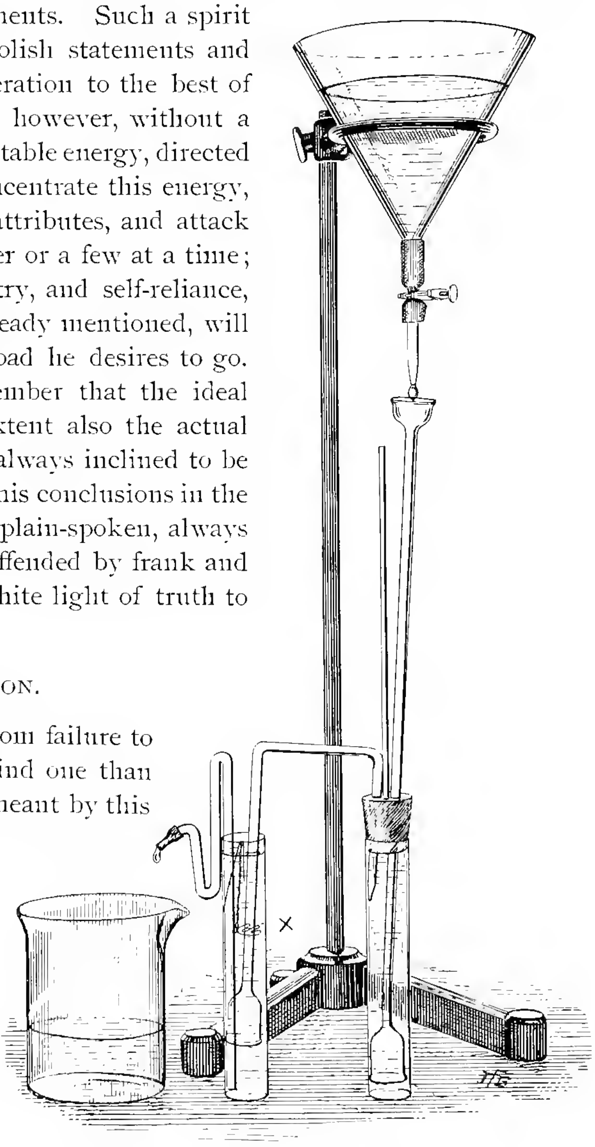

Fig. 146.*

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*FIG. 146.—Apparatus for removing water from tissues with a minimum of injury. The specimen is placed on the wire carrier at X in water. The tube at the right also contains water. Alcohol (95 per cent) is then poured into the funnel and allowed to pass into the apparatus drop by drop. Its perfect diffusion through the water is obtained by making the basal ends of the carrying tubes flaring or funnel-shaped. By gaging the time between drops the alcohol may be substituted for the water, slowly or rapidly, in any desired time. About one-third actual size.

ference is that this tube of potato-broth, which is only the third remove from the colony, contains a pure culture of the organism with which I started, but simple observations of the tube, even when coupled with a very firm persuasion, do not *assure me* that such is the fact. I check the inference by making plate-cultures and find in the tube either (*a*) only the original organism; (*b*) a mixture of two or more organisms; (*c*) a pure culture of some wholly different organism, which entered during one of the transfers as an accidental contamination and has crowded out the original organism.

(2) A plant is inoculated from a solid culture or fluid culture of a supposed parasite, and becomes diseased. The inference is that the inoculated organism has caused the disease. I check this inference by making plate-cultures from the interior of the diseased tissues and find (*a*) great numbers of the inoculated organism in pure culture and capable of again producing the disease, which I determine by actual experiment; (*b*) a mixture of organisms; (*c*) some wholly different organism; (*d*) no bacteria whatever.

(3) Fermentation-tubes of cane-sugar bouillon inoculated with a supposedly pure culture soon show clouding in the closed end, with an abundant production of gas and acid. The inference is that these phenomena are due to the presence of this particular organism. I check this inference by making plate-cultures and find (*a*) a pure culture of the original organism; (*b*) only an intruder; (*c*) a mixture of two organisms, in which case both may break up the sugar in the manner described, or only one of them.

(4) Drops of fluid containing a supposedly pure culture are dried on sterile cover-glasses and subsequently put into sterile beef-broth, which becomes clouded. The inference is that the organism in question has resisted the drying. I check this inference by making plate-cultures from the fluid and find (*a*) a pure culture of the right organism; (*b*) a pure culture of some intruder.

(5) The thermal death-point of an organism is tested by inoculating tubes of beef-bouillon and exposing them to a given temperature in the manner already described. Subsequently the bouillon clouds or does not cloud, as the case may be. The inferences are that the organism is killed or is not killed by the exposure. The first inference is checked by having at the same time inoculated other tubes of the same bouillon, which have been kept at room-temperatures, and which (*a*) do not cloud, showing either that the bouillon itself inhibits growth or that only dead organisms were inserted, *i. e.*, those from too old a culture; or (*b*) which cloud readily, showing that failure to grow in the exposed tubes is actually, as it was presumptively, attributable to the temperature of the water-bath. I check the second inference by making poured plates from the clouded tubes and find (*a*) pure cultures of the right organism; (*b*) pure cultures of some intruder.

(6) A plant, which we will designate as A, is subject in the field to a certain disease, and this disease is readily reproduced under experimental conditions, using pure cultures of a given microorganism. A related plant, which we designate as B, is subject in the field to a similar disease. A microscopic examination shows similar lesions associated with a morphologically similar organism, and Petri-dish

poured plates indicate the presence of a physiologically similar organism in both plants. The first inference is that the two diseases are caused by the same organism. A test-experiment is now instituted, viz, one or two varieties of B are inoculated with the organism obtained from A, but these do not contract the disease. An easy second inference now is that we are dealing with two distinct diseases. This may be perfectly correct, but it is not established by the experiment. Owing to an oversight, plants of A were not inoculated at the same time and in the same manner as B, to serve as checks, and consequently we are not *assured* as to the virulent nature of our culture—it may have been dead, or non-virulent, or the wrong organism. Check-plants should have been inoculated. Assuming, however, that this was done, and that A promptly contracted the disease while B remained unaffected, it is not yet certain that the disease in the two plants is due to different organisms. The question of individual and varietal resistance to disease may have entered to complicate results. To eliminate this possible source of error a greater number of varieties of B should be tested with a larger number of individuals in each variety. Cross-inoculations should also be made, *i. e.*, numerous varieties and individuals of A should be inoculated with the organism isolated from B.

Enough has been said to show the ordinary method of work. All inferences should be carefully confirmed by frequent poured-plate cultures in Petri dishes, by cultivations on the media which have been found to give most characteristic results, and, finally, by frequent inoculations into the host-plants. In case of unexpected or striking results it is always safe to determine whether they can not be obtained in the absence of the assumed cause.

These methods involve an almost endless amount of drudgery, but they are fundamental to any large success in the domain of pathology, and those who are desirous of winning a shining reputation without much labor are advised to cultivate some easier science. For those who are really in earnest, who do not mind hard work, and who have acquired the requisite training, no field affords greater opportunity for brilliant and useful work than that of plant pathology.

FORMULÆ.

When not stated the solids are reckoned in grams and the fluids in cubic centimeters. Water is understood when no particular solvent is mentioned.

STAINS.

GENERAL AND MISCELLANEOUS.

Alcoholic Solutions of Anilin Stains.

These should be saturated solutions, made preferably with Grubler's stains and absolute alcohol. In well-stoppered bottles they keep indefinitely.

Watery Solutions of Anilin Dyes.

These do not keep long and must be made up fresh each time. If made directly from the dry powder or crystals, rather than from the alcoholic solution, the resulting fluid should be passed through filter paper before using. Watery solutions are usually made by adding the alcoholic solution to distilled water in any strength desired. Usually a few drops of the alcoholic solution to 5 or 10 cc. of water is sufficient.

Anilin Water.

Anilin water is made by shaking thoroughly one part of anilin in 20 parts of distilled water and filtering it clear by passing one or more times through filter paper moistened with water. It should be prepared fresh each time. Anilin, known also as anilin oil, is a colorless, oily-looking fluid. It oxidizes to a brown color if exposed to the air, and it should therefore be kept in a close-stoppered bottle in the dark. The brown fluid is still usable, at least for some purposes.

Ziehl's Carbol-Fuchsin.

Fuchsin (basic)	1
Absolute alcohol	10
Carbolic acid (5 per cent sol. in water)	100

The fuchsin should first be dissolved in the alcohol and then the two fluids mixed. A powerful and much-used stain.

Ehrlich's Anilin-Water Gentian Violet.

Alcoholic solution of gentian violet (saturated)	5
Anilin water	100

This should be used as soon as prepared. It does not keep well.

Flexner's Anilin Gentian Violet.

Anilin oil	2
Alcohol, 95 per cent	5
Saturated alcoholic (absolute) solution of gentian violet	8
Distilled water	80

Mix well and filter.

Ehrlich-Weigert Anilin Methyl Violet.

Alcoholic solution of methyl violet (saturated)	11
Absolute alcohol	10
Anilin water	100

Does not keep well.

Anilin Fuchsin.

Prepared in the same way as Ehrlich's anilin gentian violet.

Ziehl-Nielson's Stain.

Used chiefly as a means for identifying tuberculosis. The cover-glass bearing the specimen is floated for 3 to 7 minutes on carbol-fuchsin which is heated until steam begins to appear. It is then washed in distilled water, plunged into 10 per cent nitric or sulphuric acid long enough to decolorize (a very short time). It is then passed through 60 per cent alcohol for a few seconds (just long enough to remove the stain from the background), washed thoroughly in water, dried, and mounted in balsam. The cover-glass preparation may be obtained also by dropping some of the stain upon it and holding it over the flame. This method is more economical of stain and time and less mussy than the preceding.

Friedlaender's Stain.

This has been used so far mostly for identifying the tubercle organism in sputum. It is made as follows: A few drops of carbol-fuchsin are placed on the prepared cover (which has been gently flamed) and heated over a flame until the fluid steams. The cover is then washed in distilled water, and plunged for a half minute or so into acid alcohol (c. p. nitric acid 5 cc., 80 per cent alcohol 100 cc.). It is then washed in water, stained about 5 minutes (for contrast) in an aqueous solution of methylene blue, dried, and mounted in cedar oil or balsam.

Loeffler's Alkaline Methylene Blue.

Alcoholic solution of methylene blue (saturated)		30
Caustic potash	1	100
Distilled water	10,000	

This fluid retains its valuable properties for a considerable time and is an excellent stain.

Kühne's Carbol-Methylene Blue.

(1) Methylene blue	1.5
Absolute alcohol	10.0

(2) After triturating the above in an agate or porcelain mortar, or in a watch glass, add gradually 100 cc. of water containing 5 per cent carbolic acid. Methylene blue is not the same as methyl blue. (See Pregl, Bibliog., XIV.)

Gram's Stain.

This is a method of differential bleaching after a stain. The cover-glass preparations or sections are passed from absolute alcohol into Ehrlich's anilin gentian violet or into a watery solution of methyl violet, where they remain 1 to 3 minutes, except tubercle bacilli preparations, which remain commonly 12 to 24 hours (Gram). They are then placed for 1 to 3 minutes (occasionally 5 minutes) in iodine potassium iodide water (iodine crystals 1, potassic iodide 2, water 300), with or without first washing lightly in alcohol. In this they remain 1 to 3 minutes. They are then placed in absolute alcohol until sufficiently bleached, after which they are cleared in clove oil and mounted in Canada balsam. By this method the stain is removed from some kinds of bacteria and not from others.

Too much confidence must not be placed in this method, since in some cases the removal or non-removal of the stain from the organism depends on the length of exposure to the iodine water. It would be better, therefore, to expose all for the same period, *e. g.*, 2 minutes.

Gabbett's Stain.

Used mostly for tubercle bacteria in sputum. Stain first with carbol-fuchsin, then place the cover-glass for 1 to 2 minutes in acid methylene blue (methylene blue 2 grams, 25 per cent sulphuric acid water 100 cc.). When washed in water and dried it may be mounted in cedar oil or in balsam. The ordinary bacteria of sputum are decolorized; the tubercle organism retains the red stain.

The Ehrlich-Weigert Stain.

Used for detecting the tubercle organism in sputum. The prepared cover is floated face down on anilin methyl violet, which is heated until steam rises. After 2 to 5 minutes on this hot stain plunge for a few seconds into acidulated water (1 part nitric acid, 3 parts distilled water), then wash for a few seconds in 60 per cent alcohol, and afterward thoroughly in water. For a contrast stain the cover may be placed for 5 minutes in a saturated aqueous solution of vesuvin. It is then washed in water, dried, and mounted in balsam.

Bacteria which hold the stain after such treatment are sometimes called "acid-fast" bacteria.

Flemming's Triple Stain.

The slide is first placed in (1).

(1) Safranin O (saturated alcoholic solution)	50
Distilled water	50
Anilin water	5

After washing in water, it then goes into (2).

(2) Saturated aqueous solution of gentian violet	50

It is then washed in water and passed into (3).

(3) Aqueous solution of orange G, strong or weak (generally about one-half saturated).

The slide is then washed quickly in 95 per cent alcohol, dehydrated, cleared, and mounted.

Pregl's Method.

(See '91 Pregl, Bibliog., XIV.)

Nicolle's Methods.

(See '95 Nicolle, Bibliog., XIV.)

Benda's Iron Haematoxylin.

Mordant the sections for several hours in 1 part of the following ferric solution* diluted with 2 parts of water:

Ferrous sulphate	80
Water	40
Sulphuric acid	15
Nitric acid	18

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*This solution, known to the German Pharmacopoeia as Liquor ferri sulphurici oxydati and to the U. S. P. as Liq. f. tersulphatis or sol. persulphate of iron, keeps indefinitely. It is made as follows: Heat in a flask on a water-bath until fluid is brown and clear, and a drop diluted with water is no longer colored blue by potassium ferricyanide, evaporate in a tared porcelain capsule to 100 parts, add a little water and evaporate again. Repeat the dilution and evaporation until the hot fluid is free from the odor of nitric acid. Finally dilute to a weight of 160 parts.

Wash the sections in distilled water and then in tap-water. Stain (until very black) in water containing 1 per cent haematoxylin. Differentiate in 30 per cent acetic-acid water with careful watching, or in more dilute acid, or in very dilute (1 : 20) liquor ferri, if it is to be followed by acid fuchsin as a contrast stain. (Verhandl. d. Anat. Gesellsch., 1893, Jena, Gustav Fischer.)

Heidenhain's Iron Haematoxylin.

Mordant the sections from one-half hour to 12 hours in a 2.5 per cent watery solution of iron alum (ammonio-ferric sulphate $(NH_4)_2 Fe_2 (SO_4)_4$ dissolved cold). This salt comes in violet crystals. Yellow or green crystals should be rejected. Rinse well in water. Stain in water containing 0.5 per cent of haematoxylin. Rinse, expose again to the iron-alum solution, watching the differentiation under the microscope, the examination being made in tap-water. When properly differentiated wash 15 to 45 minutes in running water. Dehydrate, clear only with xylol, and mount in xylol balsam. The mordant does not keep indefinitely, but is said to retain its properties for some time (Dodge).

Schaffner's Safranin Picro-nigrosin.

The slides are stained for a few minutes in anilin-safranin made as follows.

(1) Anilin water..................	50
Saturated alcoholic (95 per cent) solution of safranin....	50

Rinse quickly in water. They are then stained in (2).

(2) Distilled water................	100
Picric acid thoroughly dissolved in the above.................	1
Then add nigrosin............	1

Rinse in water, wash rapidly in 95 per cent alcohol, dehydrate, and mount in balsam

Malachite Green.

For plant tissues, as well as animal tissues, this may be used as a contrast stain, following carbol-fuchsin. It is dissolved in anilin (1 : 1000) and used fresh; generally the exposure to it should be for only a very brief period, *i. e.*, 1 to 3 minutes. If not fresh, much longer exposures are required.

CLEANING COVER-GLASSES FOR FLAGELLA STAINS.

Van Ermengem recommends boiling in a mixture of water 100 cc., concentrated sulphuric acid 60 cc., potassium bichromate 60 grams. The covers are afterward thoroughly washed, first in water and then in absolute alcohol. They are set on edge and dried under a bell-jar.

Loeffler recommends heating covers in concentrated sulphuric acid. They are then washed in distilled water and put into alcohol-ammonia, from which they are wiped with a very clean linen cloth.

Covers cleaned in the ordinary way may be freed from fat by passing them through a Bunsen flame immediately before using.

FLAGELLA STAINING.

There is no easy road to success. Some of the common stones of stumbling are (1) oily or otherwise dirty covers; (2) cultures unsuited either by age or by composition of the medium; (3) the casting off of flagella on dilution or during the slow drying of the fluid on the cover; (4) an uneven or too copious distribution of the organisms on the cover; (5) imperfect mordanting; (6) excessive mordanting; (7) understaining; (8) overstaining; (9) precipitates on the cover-glass during some stage in the process.

If clean covers are used, if the bacteria are derived from young moist agar cultures, if a very small quantity of such culture is put into a large drop of well aerated water, or, better, into a test-tube or watch-glass containing 5 or 10 cc. of water, and a tiny quantity of this dilution is taken on a platinum needle and deftly swept over the whole cover; or if the needle is touched to the bacterial fluid and then touched to one or more parts of a large drop of water on the cover, which is then put into the thermostat, so that it shall dry quickly and yet allow time for various rods to swim free from the tangle of their fellows ; if the mordanting is thorough, but not excessive, and, finally, if there is no unforeseen mishap in the subsequent staining, good preparations of many organisms are easily secured. Others have given the writer much trouble (*Bacillus amylovorus* is one of the worst), and the only conclusion he has been able to reach is that the bacteria vary greatly in their response to flagella stains. Sometimes well-cleaned covers give trouble and then the surface of the glass itself is at fault (V. A. Moore), and covers of another origin should be tried.

Young agar cultures are usually preferred, *i. e.*, those not over twenty-four hours old, but good results may occasionally be obtained from much older cultures and also from other media. In case flagella are to be stained from fluid cultures by ordinary methods, the medium in which the organism is grown must be very *dilute*, and there must usually be an additional extensive dilution on the cover, or before putting thereon, to avoid a dense ground stain. Bouillon contains too many fine particles, but Zettnow has found a way to stain from bouillon (Bibliog., XII).

Recently the writer has obtained very good results with several organisms by growing them for some days in 10 cc. of distilled water to which 2 or 3 drops of Uschinsky's solution was added. Covers were prepared directly from this solution. Others have reported good success by transfer of agar-grown organisms to considerable water in a watch-glass or test-tube and incubating this in the thermostat for some hours before preparation of the covers. Others have recommended the use of filtered, sterile, hydrant water instead of distilled water. Nearly every worker has some favorite stain. The writer prefers Van Ermengem's.

Loeffler's Flagella Stain.

(1) Mordant:

Solution of tannin (20 per cent in water)....	10
Saturated (cold) aqueous solution of ferrous sulphate*	5
Saturated alcoholic solution of basic fuchsin.	1

(2) Stain: Carbol-fuchsin.

(3) Corrective solutions: (*a*) 1 per cent solution of caustic soda; (*b*) a sulphuric-acid solution of equivalent strength.

V. A. Moore's Flagella Stain.

This is a slight modification of Loeffler's.

(1) Mordant:

Tannic acid (20 per cent in water)..........	10
Ferrous sulphate (cold saturated water solution)	5
Basic fuchsin (saturated alcoholic solution).	1

(2) Stain: Ziehl's carbol fuchsin.

Use the mordant fresh, and filter each time before using. Fix the film on the cover-glass by passing it quickly face up twice through the open flame, or by exposing for 5 or 10 minutes to a temperature of 120° to 140° C., the better way. A few cubic centimeters of the mordant are now placed in a wide test-tube (1 inch), the cover is lowered into it, and the mordant is heated over a flame until steam rises. It is then removed from the flame and the cover is allowed to remain in the hot fluid 5 or 10 minutes, with occasional gentle shaking, after which it is drawn to the mouth of the tilted tube by means of a hooked platinum wire set into a glass rod. After thoroughly rinsing under the tap or in a fine stream of water the cover is lowered into the stain (held in another wide test-tube), where it is heated until steam rises (1 to 3 minutes). One should know from beginning to end which side of cover bears the bacterial film. The cover is drawn to mouth of tube by means of a hooked platinum wire. Sometimes 1 per cent sol. NaOH ($\frac{1}{2}$ cc.) may be added to mordant with advantage.

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*The iron oxide may be removed from the solution of ferrous sulphate by passing it through a filter paper. Selected crystals should be used.

Fischer's Flagella Stain.

This is a slight modification of Dr. Cörner's, which is itself a modification of Loeffler's.

(1) Mordant:

Dry tannin	2
Water	20
Solution ferrous sulphate (1 : 2)	4
Concentrated alcoholic solution basic fuchsin	1

Filter This mordant will keep for several weeks. Drop it on the cover and heat over a gentle flame until steam rises; then continue one-half minute longer without boiling. Wash. Treatment with alcohol is unnecessary.

(2) Stain: Place on the cover a few drops of concentrated watery solution of basic fuchsin. Heat slowly, so that steam rises after about 1 minute. Then expose for one-half minute longer, so that the stain boils up once or twice. Wash, dry, and mount. Fischer says tannin absorbs moisture readily, and advises keeping it in a desiccator.

Bunge's Flagella Stain.

(1) Mordant:

Tannin 20, water 100	30
Liquor ferri sesquichlorati	1
Water	20
Saturated watery solution basic fuchsin	5

This must ripen some weeks by exposure to the air in a flask loosely plugged with cotton.

(2) Stain: Carbol-fuchsin.

Expose to the filtered mordant 5 minutes, using heat if necessary. Wash and stain.

Van Ermengem's Flagella Stain.

This is made as follows:

(1) Mordant:

Osmic acid (2 per cent water solution)	50
Tannin (10 to 25 per cent in water)	100

Four drops of glacial acetic acid may be added to this.

(2) Silver bath: 0.25 to 0.5 per cent nitrate of silver dissolved in distilled water in a very clean bottle.

(3) Reducing and strengthening bath:

Gallic acid	5
Tannin	3
Fused sodium acetate (some books say fused potassium acetate)	10
Distilled water	350

The flamed cover-glass (it may be unflamed) is first covered with the mordant for one-half hour, or if in a thermostat at 50° C. for 5 or 10 minutes. The mordant is then carefully removed by thorough washing in water, alcohol (some say absolute alcohol), and water. The cover (film side up) is now put into the silver bath (a few cubic centimeters in a small, perfectly clean beaker or watch-glass) for a few seconds, during which it is gently agitated. Without rinsing, it is put next into a few cubic centimeters of the reducing solution and gently agitated until the fluid begins to blacken. It is then washed in water and examined. If not stained deeply enough the cover is returned to the silver bath, then once more passed through the reducing bath. It is finally dried and mounted in balsam All the dishes must be scrupulously clean. The fluids must not be contaminated by the fingers nor by dipping iron or steel instruments into them.

Kuntze has suggested some improvements. (See Centralb f. Bakt., I Abt., Bd. XXXII, 1902, pp. 555-560.)

Pitfield's Flagella Stain.

(1) Mordant:

Tannic acid, 10 per cent aq. sol.	10
Corrosive sublimate, sat. aq. sol.	5
Alum, sat. aq. sol.	5
Carbol-fuchsin	5

Heat on cover until steam rises; keep at this temperature 1 minute; then wash, dry, and stain.

(2) Stain:

Alum, sat. (cold) aq. sol.	10
Gentian violet, sat. alc. sol.	2

Kendall, in Journ. Applied Micr., Vol. V, 1902, p 1836, says this has proved a very satisfactory stain to himself and his associates.

Another formula is given as follows:

(1) Saturated (cold) aqueous solution of alum	10
Saturated alcoholic solution of gentian violet	1
(2) Tannic acid (tannin)	1
Distilled water	10

A mixture of these two fluids is put on the flamed cover, which is held over the flame and gently heated until nearly ready to boil. The cover is then put aside for 1 minute, after which it is washed in water, dried, and mounted in balsam. If the mixed mordant-stain is filtered before using, it is best to stain a second time for a moment in anilin-water gentian violet.

Löwit's Flagella Stain.

Löwit's modification of Loeffler's flagella stain consists in substituting a copper-tannin mordant for one of iron-tannin. It is made as follows:

(1) Mordant:

Distilled water	10
Tannin	25

Dissolve and filter through two thicknesses of filter paper, then add:

Saturated solution copper sulphate	5
Saturated alc. sol. basic fuchsin	1

Filter as before.

Covers are exposed to this mordant 20 seconds to 3 minutes without heating. Wash thoroughly.

(2) Stain: Expose cover to Ehrlich's anilin water gentian violet 1 to 5 minutes. Wash thoroughly in water, and if this is not sufficient plunge for a moment into 50 per cent alcohol or into acid alcohol (1 drop of 0.3 per cent HCl alcohol in 3 to 4 cc. of 60 per cent alcohol). The mordant and stain must be made up each time. If the mordant has been in use for some hours so that an oxidation film has formed on its surface, it is well to stop and remove this by filtration.

Sclavo's Flagella Stain.

Sclavo exposes some minutes in the mordant (tannin 1, water 50, alcohol 50); washes in aq. dest.; exposes some minutes in 50 per cent phospho-tungstic acid; washes carefully in aq. dest.; stains 3 to 5 minutes in gently warmed anilin-water fuchsin; washes, dries, and mounts in Canada balsam. Some kinds are not stained by this method.

Bowhill's Flagella Stain.

This author used two solutions made as follows:

(1) Orceïn	1
Absolute alcohol	50
Distilled water	40
(2) Tannic acid (tannin)	8
Distilled water (hot)	40

Equal parts of (1) and (2) are mixed and filtered. Bacteria from a fresh agar culture are suspended in boiled distilled water. This suspension is allowed to stand 5 minutes. Drops are taken and spread and dried on clean covers, which are then taken in the fingers and fixed over the flame. They are now floated, film down, on the mordant (gently warmed) for from 10 to 15 minutes, then washed in water and dried. Ehrlich's anilin-water gentian violet is now dropped on the cover, and this is heated over the flame until steam appears. The preparation is then washed, dried, and mounted in xylol balsam.

Subsequently Bowhill modified the above as follows, using the orceïn itself as a stain:

(1) Saturated solution of orceïn (allowed to ripen about 10 days).

(2) 20 per cent solution of tannin dissolved in hot water.

The stain is prepared by taking of No. 1, 15 cc.; No. 2, 10 cc.; and distilled water, 30 cc. After mixing, the fluid should be filtered. Subsequent bleaching of the preparation should be avoided. (Hyg. Rundsch., VIII Jahrg., 1898, pp. 11 and 105.)

Hinterberger's Method for Flagella.

(See '00 Hinterberger, Bibliog., XII.)

Night Blue Stain for Flagella.

(See '99 Morton, Bibliog., XII.)

Zettnow's Method for Flagella.

Zettnow fixes with formalin, mordants with tartrate of antimony and tannin, and stains with gold or silver.

The fixing is done by taking bacteria from a fresh agar or bouillon culture and adding them to water. They are then killed by the addition of 4 per cent formalin. The fixed bacteria settle after a day or two, when the sediment containing them is pipetted out and washed, first in 1 per cent formalin water and finally in pure water. The cloudy water is spread and dried on clean covers, and when these have been fixed by gentle heat they are ready for the mordant. The mordant is made as folows:

(1) Tannin	5
Distilled water	100

Flask and heat to 35° or 40° C. in the water-bath.

(2) A solution of tartrate of antimony (1 gram dissolved in water in a test-tube) is added drop by drop to solution (1), with shaking until the precipitate which forms is not redissolved. It is then filtered. The filtered mordant should be strongly opalescent, but not cloudy or opaque to transmitted light. This is said to be a permanent universal mordant, and one which does not cause precipitates on the cover. It is used hot (70° to 80° C.) for 5 or 10 minutes. The cover is then washed and gilded or silvered. Afterward the image may be intensified if desired.

The gold method consists of placing on the mordanted cover 4 or 5 drops of an aqueous solution of neutral gold chloride (1 : 2000). This is then heated until steam is given off freely. If the mordanting has been sufficient there will be a deposit of metallic gold on the bacteria.

Hugh Williams' Method.

(Copied from Mallory & Wright's Pathological Technique.)

This is a modification of van Ermengem's method along the lines of the modification of Hinterberger and others. It has been adopted by Dr. Hugh Williams after a large experience with various methods in the laboratory of the Massachusetts General Hospital.

The method is capable of giving black bacteria and flagella, with little or no precipitate. The method is as follows:

(1) Cover the cover-glass with a mordant consisting of—

Alumnol,* 1 per cent solution...	1 part
Osmic acid, 2 per cent solution..	1 part
Tannin, 20 per cent solution.....	3 parts

Shake the mixture, add three drops of glacial acetic acid, and again shake.

(2) Apply the mordant less than one minute without heating. Wash thoroughly in water.

(3) Cover the preparation, during about 1 minute, with a 1 per cent solution of silver nitrate to which sufficient ammonium hydroxid has been added to keep the silver in solution.†

(4) Wash in water.

(5) Wash with 0.6 per cent solution of sodium chlorid.

(6) Flood the preparation with a 30 per cent solution of ammonium hydroxid, and immediately wash in water.

(7) Apply a few drops of Ortol photographic developer. (The directions for making up this developer come with the Ortol.)

(8) Wash in water.

(9) Cover with a 1 per cent solution of gold chlorid during a few seconds.

(10) Wash in water and apply Ortol developer for a few seconds.

(11) Wash in water and cover with a 1 per cent solution of mercuric chlorid for a few seconds.

(12) Wash in water.

(13) Apply Ortol developer for a few seconds.

(14) Wash in water and repeat the application of chlorid of gold, the washing, and the application of the developer two or more times. Between the various applications of the chlorid of gold the preparation should be inspected with a high, dry lens to determine the progress of the staining. This is readily done by placing the cover-glass, charged side upward, on a slide. In this way the process of impregnation with gold may be controlled; for the flagella, if stained, may be easily seen with the high-power dry lens.

The preparation is very conveniently held during the process in cover-glass forceps. The washing is best done in a small stream of water from a faucet. The various solutions are conveniently applied from dropping-bottles.

It will be seen that the process consists essentially in the impregnation of the flagella with silver, followed by intensification, in the photographic sense, with mercury and gold. The object of the application of the sodium chlorid and ammonia is to remove the excess of silver compounds which adhere to the surface of the cover-glass in spite of washing. This excess of silver compounds is chiefly responsible for the precipitates which appear on the preparation after the intensification. In spite of the application of the sodium chlorid and ammonia solutions, some precipitate will occur if the intensification is pushed too far. On this acount it is advisable to observe the progress of the intensification under the microscope, as above indicated.

Although this method may appear complicated, in practice it requires but a few minutes to stain a preparation.

Duckwall's Method.

Streaks are made on 2 per cent agar in Petri dishes from young growths in bouillon. Suspensions are made in water according to nature of organism, the motile bacteria being divided into six groups for staining purposes. Pigments and slime are removed by shaking with chloroform. Cover-glasses must be absolutely clean. Mordant must be used only when fresh; dye must be fresh and used warm (Loeffler's stain); streaks on cover-glass should not be confluent. Fix without injury to flagella; stain without overheating; wash in alcohol and water without breaking film; clear in xylol; mount in xylol balsam without previous examination. For details respecting method of making suspensions see The Canner and Dried Fruit Packer, Vol. XX, Feb. 16, 1905, p. 23.

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*Farbwerke vorm. Meister Lucius u. Brüning, Höchst a. M., Germany.

†Workers in the Bureau of Animal Industry U. S. Department of Agriculture state that too great an excess of ammonia in the silver nitrate may interfere with the working of the method.

CAPSULE STAINS.

Ribbert's Method of Staining Capsules.

Water	100
Alcohol	50
Glacial acetic acid	12.5

Warm and add dahlia to saturation. The covers are barely touched to this stain, and are then washed in water. The cover may then be mounted in glycerin or balsam. The stain keeps well. If the cover is left on the stain too long the capsule becomes deep blue and can not be distinguished from the body of the organism.

Friedlaender's Capsule Stain.

(See '85 Friedlaender, Bibliog., XIII.)

Richard Muir's Capsule Stain.

(1) Mordant:

Mercuric chloride (sat. aq. sol.)	2
Tannin (20 per cent in water)	2
Potash alum (sat. aq. sol.)	5

The dried films are mordanted 2 minutes. They are then washed in water, in alcohol, and again in water.

(2) Stain: Carbol-fuchsin 2 to 3 minutes, with gentle heat.

Wash with water; re-mordant 2 to 3 minutes; wash again with water.

(3) Counter-stain: Methylene blue (sat. aq. sol.) 2 minutes. Bleach in methyl alcohol, clear in xylol.

Welch's Capsule Stain.

Fix in glacial acetic acid. After a few seconds pour off the acid and flood with anilin-water gentian violet; repeat this operation until all acid is removed; wash and examine in salt solution (0.85 to 2 per cent).

Kaufmann's Method.

Stain 2 hours at 35° C. in Loeffler's methylene blue; wash in water containing caustic potash or soda (1 : 1500), dry; expose 2 minutes in ½ per cent silver nitrate; wash again in the alkaline water; counterstain 30 seconds in basic fuchsin water (1 cc. sat. alc. sol. in 20 cc. aq. dest.); expose again to the alkaline water (seconds); dry and mount. Best adapted to demonstration of capsules in fresh tissues. The bacterial body is blue and the capsule red.

Moore's Night-blue Capsule Stain.

(See '99 Moore, Bibliog., XIII.)

Boni's Method.

Mix white of 1 egg, glycerin 50 cc., formalin 2 drops; shake well and filter. The bacteria are placed in a drop of this fluid, spread, and heated until the glycerin has entirely evaporated. Stain 20 to 30 seconds in carbol-fuchsin, wash in water, dry and counterstain 4 to 6 minutes in Loeffler's methylene blue, wash in water, dry, and mount in Canada balsam. The background is red, body of organism blue, and periphery colorless. (See '00 Boni, Bibliog., XIII.)

SPORE STAINS.

Hauser's Spore Stain.

Pass cover-glass quickly three times through flame. Drop on carbol-fuchsin and heat for 5 minutes over flame, renewing the stain as it boils away. Nearly decolorize in dilute sulphuric or acetic acid (5 per cent). Wash very thoroughly. Counterstain with a dilute watery solution of methylene blue or with Loeffler's methylene blue (Festschrift für Zenker).

Möller's Spore Stain.

(See '91 Moeller, Bibliog., XI.)

Neisser's Spore Stain.

The cover is floated on hot anilin-fuchsin for an hour. The temperature should be near the boiling point. The cover-glass is then washed in water and decolorized in acid alcohol (1 part hydrochloric acid, 3 parts alcohol). Care must be taken not to expose to the acid alcohol too long; otherwise the color will be removed from the spores also. The cover is now stained for contrast in a saturated aqueous solution of methylene blue.

Fiocca's Spore Stain.

The prepared cover is placed in a watch-glass or test-tube containing 20 cc. of 10 per cent ammonia (water solution) and 10 to 20 drops of alkalin methylene blue or other alkalin solution of anilin color. Then the fluid is heated to the giving off of steam and left for 3 to 15 minutes. It is now passed for a moment (?) through 20 per cent nitric or sulphuric acid, then thoroughly washed in water and stained for contrast, if desired, in an aqueous solution of vesuvin, malachite green, or safranin (saturated?). (See '93, Fiocca, Bibliog., XI.)

(For other methods, *e. g.*, Foth's, Klein's, Aujeszky's, see Bibliog., XI.)

NON-SYNTHETIC CULTURE MEDIA.

Standard Peptonized Beef-Bouillon.

Standard peptonized beef-bouillon is made as follows: To 500 grams of finely minced lean beef add 1,000 cc. of distilled water. The soluble parts may be removed from the meat by allowing the water to stand on it for 24 hours in the ice-chest or for 1 hour in the water bath at 55° C. The writer prefers the second method. Then boil for 60 minutes either in the steamer or in a covered dish. Filter through a clean cloth, using pressure (meat-press), cool, and remove fat by filtering through S. & S. filter paper; make up to 1,000 cc. by addition of more water; then add 1 per cent Witte's peptonum siccum and 0.5 per cent c. p. sodium chloride. Steam one-half hour, filter, cool, titrate, add required alkali, steam again for one-half hour, filter, pipette into tubes or flasks, and autoclave or heat for a minimum time in the steamer. Plugs should be well made and fit tightly; glassware should be scrupulously clean. For some purposes both the peptone and the salt may be omitted. A greenish bouillon indicates insufficient boiling, and will usually throw down some additional vexatious precipitate when heated in the test-tubes. Other meats may be substituted for beef, and other peptones for Witte's. Meat-extracts are not recommended. Such extracts usually contain resistant spores. Media which have been steamed, or boiled in an open dish, are better for many bacteria than those which have been sterilized in the autoclave.

(For additional observations on proper sterilization see *Culture Media*, p. 29.)

Dunham's Solution.

Distilled water	1,000
Witte's peptonum siccum	10
C. P. sodium chloride	5

First recommended by Dr. Ed. K. Dunham, of New York.

Standard Nutrient Agar.

To 1,000 cc. of standard beef-bouillon add 10 grams of agar-flour, steam one-half hour, cool to 58° C.; add whites of two eggs (beaten thoroughly and neutralized to litmus by dilute hydrochloric acid) and thoroughly mix with the bouillon; steam 1 hour, filter hot through S. & S paper which has been thoroughly warmed with boiling distilled water. Use two or three funnels. That which remains unfiltered after a reasonable time must be reheated and put through a fresh filter paper. Sometimes all can be got through a second filter paper without reheating. Some advise filtering in the autoclave or in the steamer, but the writer has not found that necessary, and in recent years has also abandoned the hot-water funnel.

Clear agar may be obtained also by filtering through absorbent cotton, and some prefer this to filter paper.

In preparing agar from the "slender kanten" or the "square kanten," snip fine, soak in the bouillon 15 minutes, and then heat on the sand bath 1 hour at 110° C. or in the autoclave 45 minutes at 105° C. From this point proceed as before.

Long heatings in the autoclave at 110° C., or shorter heatings at higher temperatures, are apt to brown the agar, and should be avoided carefully, as this renders the medium less serviceable for the growth of bacteria. Agar which has been properly superheated filters readily. One per cent agar made from the agar-flour does not require to be heated on the sand-bath or in the autoclave, but filters satisfactorily after steaming for an hour at 100° C.

After the agar has been tubed it may be sterilized, if it does not contain sugars, by one steaming in the autoclave for 10 minutes at 110° C, or by short steamings in the steam sterilizer at 100° C. on three successive days.

To those who are dependent on the agar-strips and do not have access to an autoclave, Schutz's method may be recommended as very good. The writer formerly made large use of this. It consists in heating the agar very hot in a minimum quantity of water or bouillon before adding the bulk of the fluid. (See p. 34 and Bibliog., XVI.)

Agar which has been soaked in 5 per cent acetic acid for an hour or two before adding it to the bouillon also enters into solution thoroughly and filters well after a short boiling. The acid must first be removed completely by washing in running water for some hours under a mosquito-net or a piece of gauze.

Unfiltered agar does well enough for certain fungi, and for lazy people, but the agar used for delicate work in bacteriology should be as clear as the bouillon from which it is made., *i. e.*, perfectly free from cloudiness and precipitates. Sufficient caustic soda is usually added to the agar to render it + 15 of Fuller's scale. Other degrees are useful, *e. g.*, + 10, 0, — 10, etc.

Litmus Lactose Agar.

To 1,000 cc. of ordinary agar, preferably that made up with bouillon free from muscle-sugar, add 10 grams of c. p. lactose and 20 cc. of a saturated (water) solution of c. p. (lime-free) blue litmus.

Hesse and Niedner's Nutrient Agar for Water Bacteria.

Distilled water..................	980
Nährstoff Heyden (an albumose)	7.5
Agar-agar	12.5

This agar is said to be the most suitable medium for the bacteriological examination of water. It gives a much larger number of colonies than ordinary agar. It requires no neutralizing. The poured plates are counted, according to Dr. Robin, on the 9th or 10th day. Chromogenic species are brilliantly colored. (Zeitschr. f. Hygiene, Bd. XXIX, pp. 454-462. See also Am. Jour. Pharm., Vol. LXXVI, p. 112.)

Glycerin Agar.

To each 1,000 cc. of ordinary agar add 50 cc. of Schering's c. p. twice-distilled glycerin.

Standard Nutrient Gelatin.

To 1,000 cc. of sterile standard peptonized beef-bouillon add 100 grams of best quality gelatin. Soak 2 hours at room-temperature. Then steam 5 minutes, cool, titrate, add the necessary alkali, steam 30 minutes, filter through S. & S. paper washed with sterile boiling hot water, tube at once, and heat in the steamer on three successive days 15 minutes, 10 minutes, and 5 minutes, respectively, at 100° C. Do not autoclave, and carefully avoid long heatings in the steamer. Have all the glassware sterile and the fluids sterile and sufficiently boiled to begin with. Very acid gelatin should be avoided. The very best English, French, and German gelatins should be used. + 10 or + 15 is a good degree of alkalinity for many purposes. Zero of Fuller's scale is also useful. See remarks on gelatin (p. 30).

Blood Serum.

(See p. 48.)

Plant Juices.

(See p. 41.)

Solid Vegetable Substances.

(See page 39.)

Milk.

(See p. 46.)

Litmus Milk.

Litmus milk is prepared from fresh milk which has been passed through a separator (centrifuge), or from milk which has stood 18 or 20 hours at 20° C. and has had the cream removed by skimming and filtration. To each 100 cc. of this milk is added 2 cc. of a saturated solution of high-grade, lime-free, blue litmus (litmus 1 gram, water 15 cc.). This gives a lavender color of just the right degree, which reddens distinctly under the action of acids, and blues with the development of alkalies. The milk selected should not titrate more than + 16 with phenolphthalein and caustic soda. A good quality often gives + 10 to + 14. High readings denote the excessive multiplication of lactic acid bacteria. Such milks frequently coagulate on steaming, and are not suitable for culture-media. After adding the litmus water the milk should be pipetted in 10 cc. portions into cotton-plugged test-tubes and heated in streaming steam (100° C.) for 15 minutes on each of 4 successive days.

This is a very useful culture medium. Every organism should be tested in it. All milk used for culture media should be centrifuged, if possible, *immediately* after milking, and secured *at once* for the laboratory. Three steamings are then sufficient. Milk offered for sale in cities is frequently more than 48 hours old and often contains from 3,000,0000 to 6,000,000 bacteria per cubic centimeter. Such milk is not fit for laboratory use.

Nutrient Starch Jelly.

The writer makes this as follows: To 10 cc. portions of modified Uschinsky's solution, or of the ordinary solution (glycerin omitted or not, as desired), is added 1 gram of clean aseptic potato starch. This is rubbed up in the slanted fluid. The test-tubes are then very tightly plugged to avoid loss of water and placed carefully in a blood-serum oven or in the top of an Arnold steam sterilizer with the vents open, where they are heated for 2 hours on each of 5 successive days at 85° C. to 93° C. If water is lost during the heating it must be made up, using a sterile pipette. Potato starch is prepared in the laboratory (p. 50) with care in the washing and drying, so as to avoid retention of other substances than starch and the multiplication of resistant (spore-bearing) bacteria, which interfere with the sterilization. (See Proc. Am. Asso. Adv. Sci., 1898, Vol. XLVII, p. 411.)

Synthetic Culture Media.

Pasteur's Culture Fluid.

Ammonium tartrate	10
Ashes of yeast	10
Rock candy	100
Distilled water	1,000

Dissolve cold.

Naegeli's Nutrient Solution.

Calcium chloride	0.1
Magnesium sulphate	.2
Dipotassium phosphate	1.0
Ammonium tartrate	10.0
Distilled water	1,000.0

Cohn's Nutrient Solution.

Distilled water	1,000.0
Acid potassium phosphate	5.0
Magnesium sulphate	5.0
Neutral ammonium tartrate	10 0
Potassium chloride	0.5

(De Bary, p. 86, Vorles. ü. Bact., 2 Auflage.)

Raulin's Culture-Fluid.

Distilled water	1,500.00
Granulated cane sugar	70.00
Tartaric acid	4.00
Ammonium nitrate	4.00
Ammonium phosphate	.60
Potassium carbonate	.60
Magnesium carbonate	.40
Ammonium sulphate	.25
Zinc sulphate	.07
Ferrous sulphate	.07
Potassium silicate	.07

Prazmowski's Culture-Fluid.

Dipotassium phosphate	5.0
Magnesium sulphate	5.0
Ammonium carbonate	5.0
Calcium chloride	.5
Distilled water	1,000.0

Dissolve cold. Any desired sugar may be added for the carbon food.

Adolf Mayer's Culture-Fluid.

(Unters. ü. d. alc. Gähr., 1870.)

Magnesium sulphate	10.0
Ammonium nitrate	15.0
Tri-basic calcium phosphate	.1
Potassium phosphate	10.0
Distilled water	1,000.0

Dissolve cold and add sugar. Add sodium chloride (3 per cent) if it is to be used for luminous bacteria, and an excess of pure carbonate of lime if acid-forming bacteria are to be grown.

Uschinsky's Solution.

Distilled water	1,000
Glycerin	30 to 40
Sodium chloride	5 to 7
Calcium chloride	0.1
Magnesium sulphate	0.3 to 0.4
Dipotassium phosphate	2 to 2 5
Ammonium lactate	6 to 7
Sodium asparaginate	3 to 4

Modified Uschinsky's Solution.

The modified Uschinsky recommended by the writer for use with starch jelly is made as follows:

Distilled water	1,000.000
Ammonium lactate	5.000
Sodium asparaginate	2 500
Sodium sulphate	2.500
Sodium chloride	2 500
Dipotassium phosphate	2.500
Calcium chloride	.010
Magnesium sulphate	.010

Fraenkel and Voges' Solution.

(Hygienische Rundschau, Bd. IV, 1894, p. 769.)

Water	1,000
Sodium chloride	5
Dipotassium phosphate*	2
Ammonium lactate	6
Sodium asparaginate	4

This paper also discusses Uschinsky's solution.

Fermi's Culture-Fluid.

Distilled water	1,000.0
Magnesium sulphate	.2
Acid potassium phosphate	1.0
Ammonium phosphate	10.0
Glycerin	45.0

This may be added to agar in place of peptonized beef-broth (De Schweinitz) or to silicate jelly, in which case the volume of water must be reduced (see Silicate Jelly, p. 36).

Moore's Culture-Medium for Leguminous Root-tubercle Bacilli.

(For field use.)

The dried culture (on cotton) is thrown into clean water containing: Cane-sugar, 1; c. p monopotassium phosphate, 0.1; c. p. magnesium sulphate, 0.01 per cent. After 24 hours add Merck's pure dibasic ammonium phosphate to amount of 0.5 per cent. Seeds are drenched with this fluid at end of another day, dried in shade, and planted.

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*Lehmann and Neumann recommend neutral commercial sodium phosphate (*l. c.* p. 29.)

Maassen's Culture-Fluid.

Malic acid	7
Distilled water	100

Neutralize to litmus exactly with 7 per cent potassium hydrate. Make up to 1,000 cc. with distilled water and add:

Asparagin	10.0
Secondary sodium phosphate	5.0
Magnesium sulphate	2.5
Sodium hydrate	2 5

When dissolved add 0.01 gram of calcium chloride.

To this may then be added grape-sugar or any other carbon food desired.

Proskauer and Beck's Culture-Fluid.

Distilled water	1,000.00
Commercial ammonium carbonate	3 50
Primary potassium phosphate	1 50
Magnesium sulphate	2.50
Glycerin	15.00

Mackensie's Culture-Fluid.

Acid ammonium tartrate	1.5
Bipotassium phosphate	2.5
Potassium sulphate	1.5
Sodium chloride	.5
Glucose	5.0
Lactose	5.0
Glycerin	15.0
Water	1,000.0

This is rendered alkalin to phenolphthalein with normal soda solution.

Culture-Medium for Luminous Bacteria.

(Molisch., *l. c.* p. 87.)

Water	1,000.000
Gelatin	100.000
Sugar	20.000
Peptone	10.000
Dipotassium phosphate	250
Magnesium sulphate	.250

Enough sodium hydroxid to render the medium feebly alkalin. On this substratum the bacteria grow feebly and are not luminous until sodium chloride or some equivalent substance is added (usually 3 per cent). Then they grow well and become luminous.

Winogradsky-Sleskin Silicate Jelly.

Ammonium sulphate	0.40
Magnesium sulphate	.05
Potassium phosphate	.10
Sodium carbonate	0.60- .90
Calcium chloride	Trace
Silicate jelly	100 00

The salts are dissolved separately in the least possible water, and added to the dialyzed acid.

(For further observations on silicate jelly see p 36.)

Nitrogen-free Medium for Bacteria.

Triple-distilled water	1,000.000
Cane-sugar	5.000
Monopotassium phosphate	2.000
Magnesium sulphate	.100
Sodium chloride	.500

All chemically pure, in scrupulously clean flasks. The water should be freshly distilled, kept in glass-stoppered bottles, and tested frequently with Nessler's solution for presence of ammonia.

Giltay & Aberson's Culture-Medium for Denitrifying Organisms.

Distilled water	1,000.0
Potassium nitrate	2 0
Asparagin	1.0
Magnesium sulphate	2.0
Citric acid	5.0
Monopotassium phosphate	2.0
Calcium chloride	.2
Ferric chloride	2 gtts.

The acid should be neutralized by the addition of potassium hydrate.

This medium is a modification of that of Gayon and Dupetit, less nitrate being used and the neutralization being made with potash instead of ammonia. In preparing this fluid the asparagin and the nitrate of potash are dissolved in 250 cc of water; the other substances are dissolved in 500 cc. of water, and after the citric acid has been neutralized the two fluids are mixed, cooled to 15° C., and sufficient water added to make 1 liter. When the nitrate of potash and the asparagin are dissolved along with the other salts a decomposition occurs, and the liquid is browned from the presence of nitrous acid, which should be avoided. Some carbonate of lime is also added to the culture fluid. Instead of asparagin, 2 grams of dextrose may be added. If the latter is used the fluid must not contain the least excess of potassa; otherwise when it is sterilized there will be more or less humification. (Recherches sur un mode de dénitrification, etc. Archives néerlandaises des Sci. Ex. et Nat., Tome XXV, 1892, pp. 341-361.)

Winogradsky's Culture-Medium for Nitrogen-Assimilating Soil-Bacteria.

Twice-distilled water	1,000.00
Potassium phosphate	1.00
Magnesium sulphate	0.50
Sodium chloride	.01 to 0.02
Iron sulphate	.01 to .02
Manganese sulphate	.01 to .02
Dextrose	20 to 40

To this should be added a small quantity of pure calcium carbonate, 30 or 40 grams per liter is sufficient. The carbonate is freshly washed in boiling water and added in paste or dried rapidly and preserved in flasks with ground-glass stoppers. It is recommended that the second distillation of the water be made with carbonate of soda and that pure salts be obtained by repeated crystallizations. It is probable that monopotassium phosphate is meant by *phosphat de potasse.* (Recherches sur l'assimilation de l'azote libre de l'atmosphère par les microbes, Archives des Science Biologiques, Tome III, p. 304, St. Pétersburg, 1895.)

Beyerinck's Agar for Cultivation of the Nitrite Bacteria.

Ordinary agar is added to distilled water, heated until it passes into solution, and poured into Erlenmeyer's flasks, where it is left to solidify. When cold the flasks are filled with distilled water (not necessarily sterile) and set away. After several changes of water and the lapse of one or two weeks the soluble organic substances will have been absorbed out of the agar, and to it may now be added the inorganic nutrient substances desired, after which it is sterilized. Along with the nutrient substances some pure precipitated calcium carbonate should be added. The sterile agar may then be solidified in Petri dishes, test-tubes, etc. Beyerinck considers this medium better for isolation of the nitrite ferment than the silicate jelly. Hydrogen ammonium sodium phosphate ($NH_4NaHPO_4+_4H_2O$) is recommended as the best one of the ammonium salts for addition to the agar, because, upon cooking, the agar is not attacked, and consequently additional soluble substances are not liberated from it. (Beyerinck: Kulturversuche mit Amöben auf festem Substrate. Centralb. f. Bakt., 1 Abt., Bd. XIX, 1896, pp. 257-267.)

Winogradsky's Nutrient Agar for Isolation of Nitrate Bacteria.

Tap-water	1,000
Agar (thoroughly washed)	15
Di-potassium phosphate	0.05
Fused sodium carbonate	1
Sodium nitrite (Merck)	2

(Centralb. f. Bakt., 2 Abt., Bd. V, 1899, pp. 537-549.)

Winogradsky & Omélianski's Fluid Culture-Medium for Isolating the Nitrate Bacteria from Soils.

Distilled water	1,000.0
Magnesium sulphate	.3
Ferrous sulphate	.4
Sodium chloride	.5
Di-potassium phosphate	.5
Fused sodium carbonate	1.0
Sodium nitrite (Merck)	1.0

Transfers through a series of flasks are necessary in order to isolate the organism. (Centralb. f. Bakt., 2 Abt., Bd. V, 1899, pp. 537-549.)

Winogradsky & Omélianski's Fluid Culture-Medium for Isolating the Nitrite Bacteria from Soils.

Distilled water	1,000.0
Ferrous sulphate	.4
Magnesium sulphate	.5
Di-potassium phosphate	1.0
Sodium chloride	2.0
Ammonium sulphate	2.0

The cultures are made in broad-bottomed flasks in 50 cubic centimeters of the fluid, to each of which about $\frac{1}{2}$ gram of magnesium carbonate is added. It is necessary to transfer through a series of flasks in order to obtain pure cultures. If the organism does not grow well on the start, additional ammonium sulphate may be added, viz, 1 cubic centimeter of a 10 per cent solution to each flask. (Centralb. f. Bakt., 2 Abt., Bd. V., 1899, pp. 537-549.)

Dubois' Fluid Medium for Luminous Bacteria.

(See '93 Dubois, Bibliog., XXVII.)

Kuntze's Medium for Bacillus Prodigiosus.

(See '00 Kuntze, Bibliog., XXIII.)

Omélianski's Magnesia-Gypsum Blocks for the Cultivation of Nitrifying Organisms.

One per cent carbonate of magnesia is uniformly mixed with gypsum and water added to it, stirring until it becomes of the consistency of sour cream, when it is poured upon plate-glass and spread out. As soon as the mass becomes of a doughy consistency and is ready to harden, it is cut into circular blocks for Petri dishes and into strips for test-tubes. The circular pieces may be cut with a Petri dish of a size a little smaller than the dishes it is intended to use. As soon as the gypsum has hardened thoroughly the blocks are pried loose from the plate glass, placed bottom up in the dishes (so as to give a smooth surface), and enough of the above (nitrite) culture-medium added to half cover the block. This is then autoclaved and additional sterile culture-media added from time to time as necessary, being careful not to wet the inoculated surface of the block. The sowings are made on the smooth surface of the block and the dishes are kept in a thermostat at 25° to 30° C. Colonies begin to be visible in 4 to 5 days. In 10 to 14 days many colonies are 0.25 to 0.50 millimeter in diameter. (Centralb. f. Bakt., 2 Abt., Bd. V, 1899, p. 652.)

MISCELLANEOUS.

Distilled Water.

(See page 124.)

Chromic Acid Cleaning Mixture.

This is made by pouring 1 gallon or more of concentrated crude sulphuric acid into an equal volume of a saturated aqueous solution of potassium bichromate. It should be done in a large enameled iron kettle, the acid being added slowly at intervals, with frequent stirring, so as to keep the mixture below the boiling point. An excess of the sulphuric acid should be avoided Pure water should be used for dissolving the potassium bichromate, and under no circumstances should this solution be poured into the acid, since steam might be generated and dangerous splutterings occur. The resulting chromic acid is very injurious to the skin and should be used with care. At 15° C. each 10 parts by weight of water will dissolve about 1 part of the potassium salt. The chromic acid mixture is said to explode violently when brought into contact with certain substances, *e. g.*, alcohol, glycerin.

Fluid for Softening Hard Tissues.

Frequently grains of cereals and other hard tissues may be softened for cutting on the microtome with slant stroke by soaking from 3 to 6 months in equal parts of alcohol and glycerin.

Unguentum resinae.

(See Bibliog., XVII, '00, Bulloch.)

Darwin's Wax-Mixture.

This consists of vaseline 50 parts, beeswax 35 parts, melted together. Then stir in of powdered resin 15 parts If a stiffer mixture is desired, add more wax up to 50 parts (see Darwin & Acton, Plant Physiology, p. 3, foot note).

Pencils for Writing on Glass.

(See page 111.)

Pyrogallol Developer.

(Much used in Laboratory of Plant Pathology.)

(1) Alkali:

Carbonate of potash (cryst.)*..........	1½ ounces,	or 46.5 grams
Carbonate of soda (cryst.)*..........	2 ounces,	or 62 grams
Distilled water......	12½ ounces,	or 375 cc.

(2) Pyro:

Sulphite of soda (cryst.)*..........	4 ounces,	or 124 grams
Citric acid..........	60 grains,	or 3.9 grams
Bromide of potash..	40 grains,	or 2.6 grams
Distilled water......	12½ ounces,	or 375 cc.
Pyrogallic acid......	1 ounce,	or 31 grams

The pyrogallol should be added last of all, and the nearly filled bottle closed at once.

For a normal developer take 2 drams of No. 1, add 2 drams of No. 2, and make up to 4 ounces with distilled water. Reduce the amount of alkali to one-fourth dram or less in case of much overexposed plates. In case of exposures likely to exhibit too great contrasts reduce the pyro. Always begin development with one-fourth of the alkali, unless the exposure is known to be correct. Both solutions should be kept in glass-stoppered bottles.

Bottles containing alkali should have the inside of the neck and the ground surface of the stopper wiped dry before replacing; then the latter will not stick.

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*In case anhydrous salts are employed, use one-half as much.

Ortol Developer.

(See pp. 140-141.)

Pyro Developer for Dry Plates.

(Recommended by S. G. Lofft.)

(1) Water	10 oz.,	or 300	cc.
Citric acid	10 grains,	or 6.46	grams
Pyrogallic acid	1 oz.,	or 31	grams
(2) Sodium sulphite (crystals)	4 oz.,	or 124	grams
Water	16 oz.,	or 480	cc.
Or			
Seed's sulphite	1½ oz.,	or 46.5	grams
Water	16 oz.,	or 480	cc.
(3) Sodium carbonate (crystals)	4 oz.,	or 124	grams
Water	16 oz.,	or 480	cc.
Or			
Seed's carbonate	2 oz.,	or 62	grams
Water	16 oz.,	or 480	cc.

To develop take—

Water	4 oz.,	or 120	cc.
No. 1	2 drams,	or 7.5	cc.
No. 2	½ oz.,	or 15	cc.
No. 3	½ oz.,	or 15	cc.

For underexposures dilute and change frequently to fresh developer.

For overexposures use old developer or restrain with a few drops of 10 per cent potassium bromide.

Lantern-slide Developer.

(Used in Laboratory of Plant Pathology.)

Distilled water	cc.	900
Carbonate of soda (cryst.)	grams	39
Sulphite of soda (cryst.)	grams	39
Hydrochinon	grams	13

Add the hydrochinon after solution of the soda salts, and put at once into a glass-stoppered bottle. For use take 3 ounces of above and 3 ounces of distilled water, to which add 5 drops of a 10 per cent solution of bromide of potassium. If properly exposed the image should appear in 30 to 60 seconds, and the development should be over in 3 or 4 minutes. Good for a dozen or more properly exposed slides.

Zettnow's Copper-chrom-filter.

Dry, pure, copper nitrate	160
Pure chromic acid	14
Distilled water	250

This may be diluted further with water if desired.

In case there is difficulty in preparing the above, a solution, stated by Zettnow to be nearly as useful, consists of—

Sulphate of copper	175
Bichromate of potash	17
Water	1,000

(Centralb. f. Bakt., IV Bd., 1888, p. 51.)

Neuhaus says, water 500 to 1,000, and also 2 cubic centimeters of sulphuric acid. This solution extinguishes all the blue and violet rays.

Toning Bath for Solio Paper.

(A) Hyposulphite of soda	8 ounces
Potash alum (crystals)	6 ounces
Cane sugar	2 ounces
Water	80 ounces

Dissolve cold, then add 2 ounces of borax which has been dissolved in 8 oz. of hot water. Let stand 12 hours, and then decant.

(B) Pure chloride of gold	7.5 grains
Acetate of lead	64 grains
Distilled water	8 ounces

This solution must not be filtered and must be shaken thoroughly each time before using.

Solio paper should be printed about one-third darker than it is desired to have the pictures. When the prints are ready they are placed face down in a toning mixture made of stock A, 8 ounces, and stock B, 1 ounce, taking care that the entire surface of each print is uniformly wetted. They are allowed to remain in this bath, with constant movement by means of the fingers, until the desired color is obtained (usually about 5 minutes). The prints are now immersed in salt water (1 : 32) for 5 minutes. They are then exposed for 15 minutes to the fixing bath, consisting of—

Hyposulphite of soda	1 ounce
Sulphite of soda (crystals)	60 grains
Borax	½ ounce
Water	20 ounces

The prints are finally washed for from 1 to 2 hours in running cold water. The toning bath should not be cooler than 40° or warmer than 60° F.

A New Test for Indol.

Herter & Foster have recently described what is stated to be a rapid and accurate method of determining indol, adapted either for colorimetric or gravimetric determinations. To slightly alkaline solutions naphthoquinon sodium monosulfonate is added. A blue crystalline compound, di-indyl naphtho-ketone mono-sulfonate is produced This is slightly soluble in water, but is readily soluble in chloroform, its solution being red. (Science, n. s., Vol. XXI, 1905, p. 987.)

FIXING FLUIDS.

Absolute Alcohol.

Expose 24 hours or more. Very useful for fixing bacteria in tissues, as it prevents their diffusion. It causes, however, considerable shrinkage of the tissues, and the nuclei are often difficult to stain, and are usually distorted. Bacteria fixed in this way stain well in Ziehl's carbol-fuchsin.

Picric Acid in Hot Absolute Alcohol.

(See p. 8.)

Mercuric Chloride in Hot Absolute Alcohol.

(See p. 8.)

Acetic Alcohol with Mercuric Chloride.

Absolute alcohol	1
Glacial acetic acid	1
Chloroform	1

Add mercuric chloride until saturated. Wash with alcohol or with alcohol containing tincture of iodine. One of the most rapid fixatives known.

Acetic Alcohol (Carnoy's Fluid.)

Glacial acetic acid	1
Absolute alcohol	3

Expose 24 hours or more and wash in alcohol This solution preserves the chromatic and cytoplasmic structures better than alcohol alone, and shrinks the tissues much less. Tissues fixed in this solution take most of the coal-tar stains better also. It has very great penetrating power, which makes it very useful in fixing large pieces of tissue.

Chromo-aceto-osmic Acid (Flemming's Fluids).

A. Stronger solution—

1 per cent chromic acid	15
2 per cent osmic acid	4
Glacial acetic acid	1

B. Weaker solution—

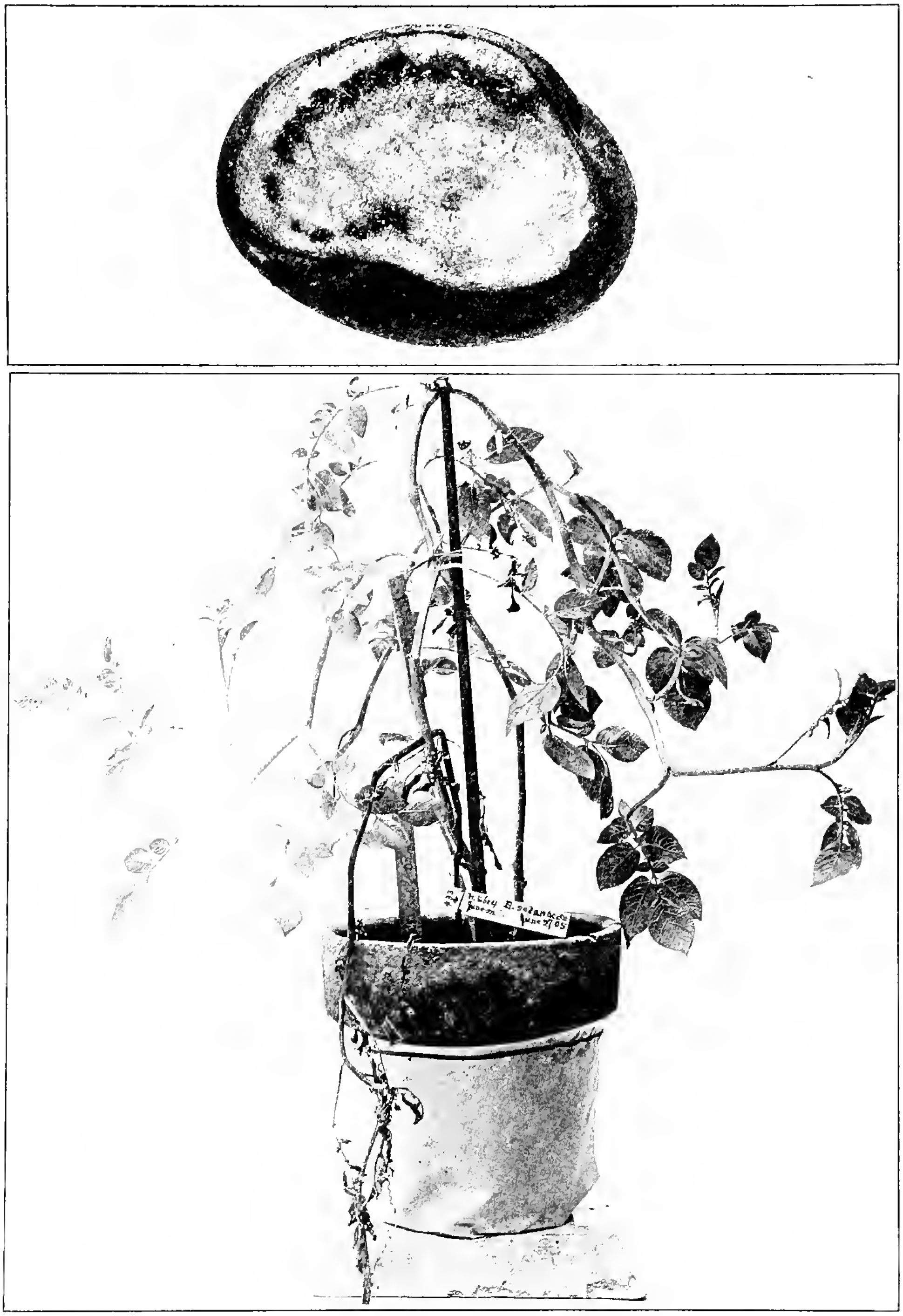
1 per cent chromic acid	25
1 per cent osmic acid	10
1 per cent acetic acid	10
Water	55

Expose 12 to 48 hours and wash in running water. This is probably the most valuable of all the fixing fluids for preserving exactly all the cell structures; and material fixed in it takes the coal-tar stains unusually well. It has very slight penetration, and therefore the tissues should be cut into pieces not more than 2 millimeters thick. It should be kept in glass-stoppered bottles, and should be made fresh every 6 months or less.

For additional formulæ consult the various standard text-books, especially Eyre's "Bacteriological Technique," where may be found, among others, the following:

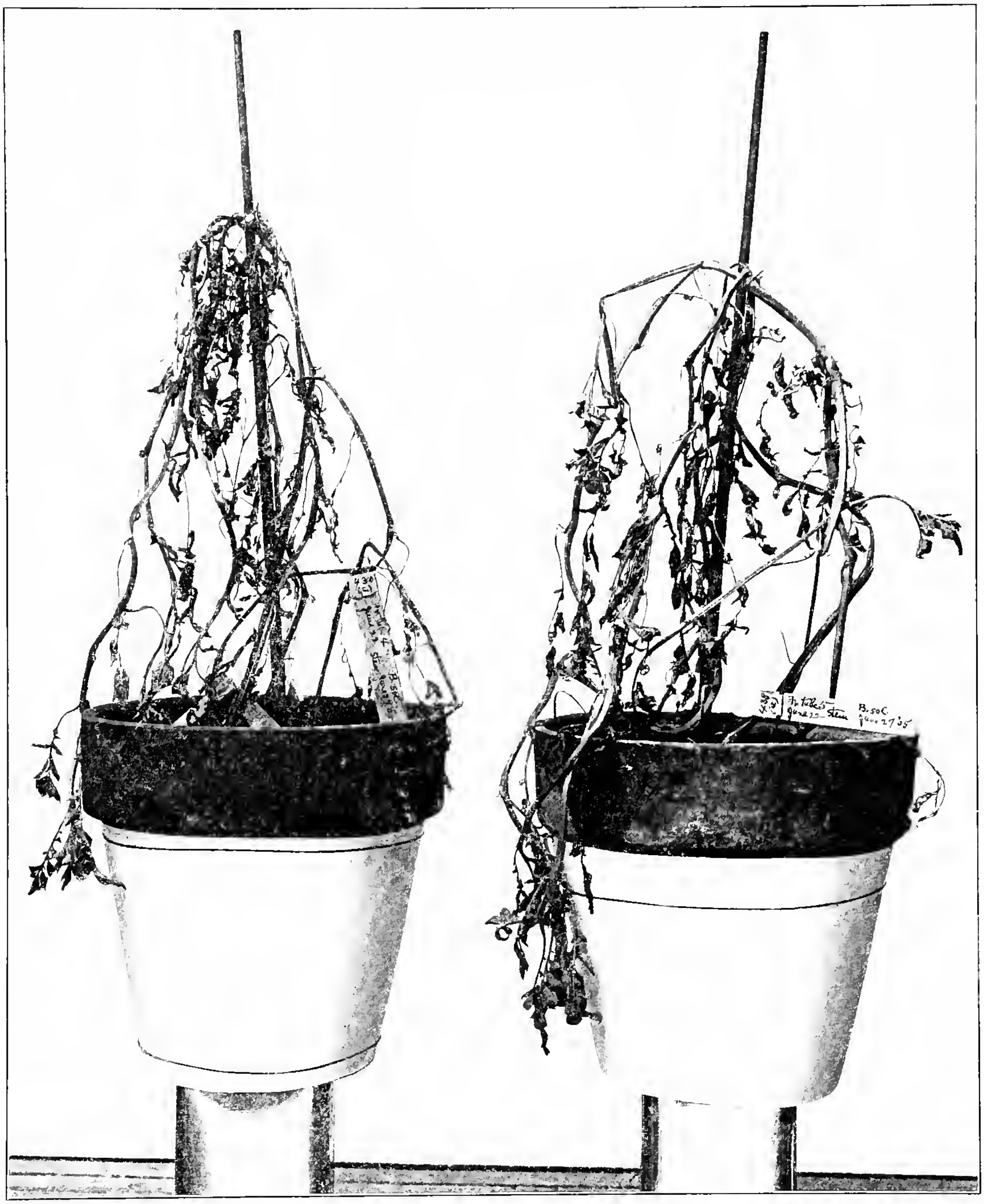
Kitasato's glucose-formate-bouillon.
Iron-bouillon. Lead-bouillon.
Parietti's bouillon.
Carbolized bouillon.
Kitasato's glucose-formate-agar.
Guarniari's agar-gelatin.
Carbolized agar.
Glycerine blood-serum.
Heiman's serum-agar.
Washbourn's blood-agar.
Urine-gelatin. Urine-agar.
Whey-agar. Fish-bouillon.
Fish-gelatin. Fish-agar.

Glycerinated potato.
Glycerine-potato-broth.
Elsner's potato-gelatin.
Goadby's potato-gelatin.
Beer-wort. Wort-gelatin.
Wort-agar. Wine-must.
Gasperini's wheat-broth.
Bread-paste. Milk-rice.
Pakes' iron-peptone-solution.
MacConkey's bile-salt-broth.
MacConkey's bile-salt-agar.
Sabouraud's French proof agar.
Blaxall's English proof agar.



Brown rot of the potato.

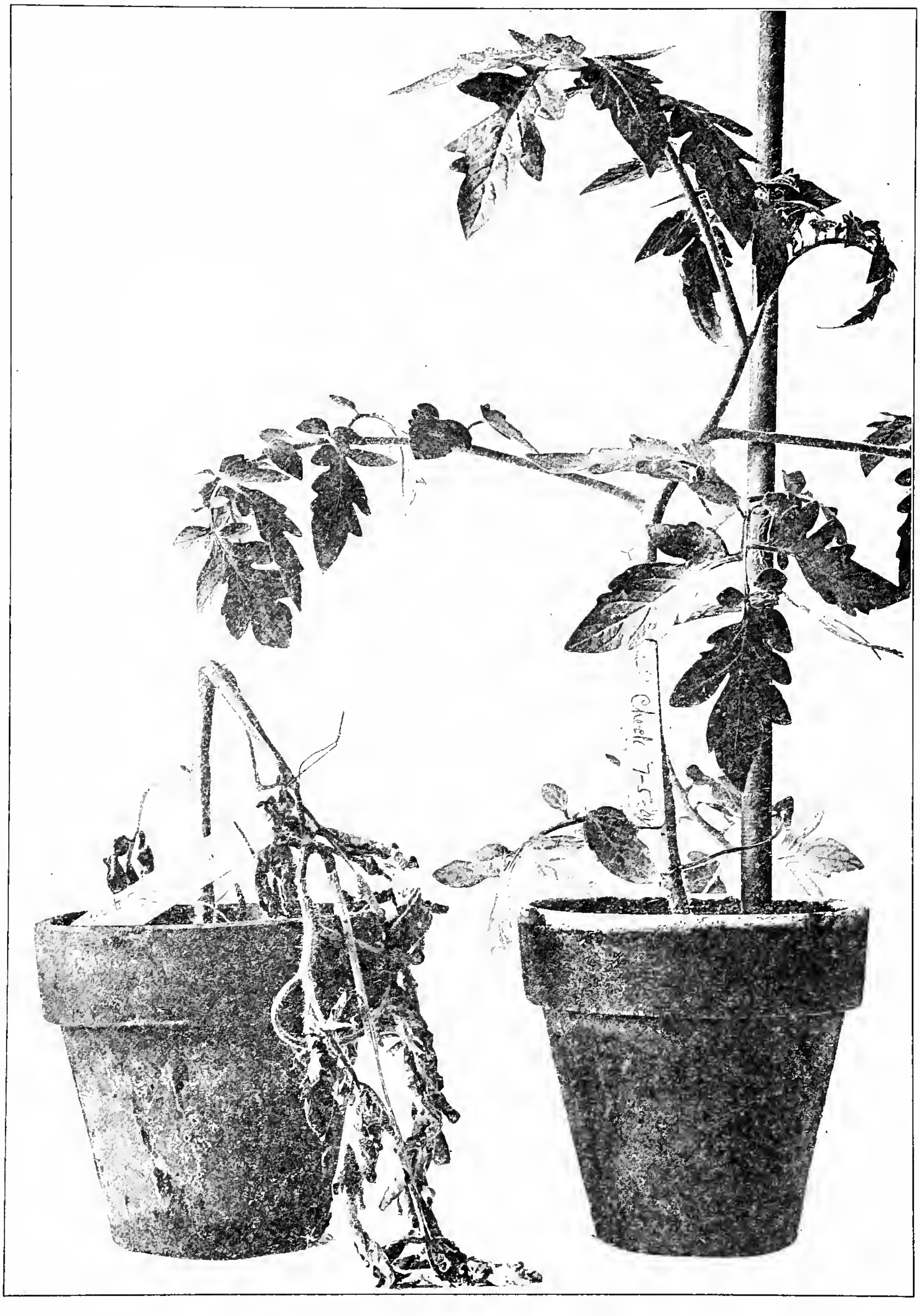
Potato-tuber from Hastings, Florida, crop of 1905, sound externally, but brown-rotted in the vascular system, the bacteria having entered through the vessels of the underground stem. Bacterium solanacearum was plated out and subcultures were used to infect potted potato-plants. The plant shown was inoculated on June 27, by delicate needle-pricks. Disease more advanced on one inoculated shoot than on the other; later this also shriveled. Tuber natural size; photographed May 29. Plant about one-fifth natural size; photographed July 15, 1905.



Early Rose potatoes destroyed by Bacterium solanacearum.

Plants inoculated at same time, in same way, and from same source as plant shown in plate 24. About one-fourth natural size. Progress of disease rather slow; no tubers were formed. All of the shoots dead or dying. Photographed August 3, 1905, i. e. at end of fifth week.

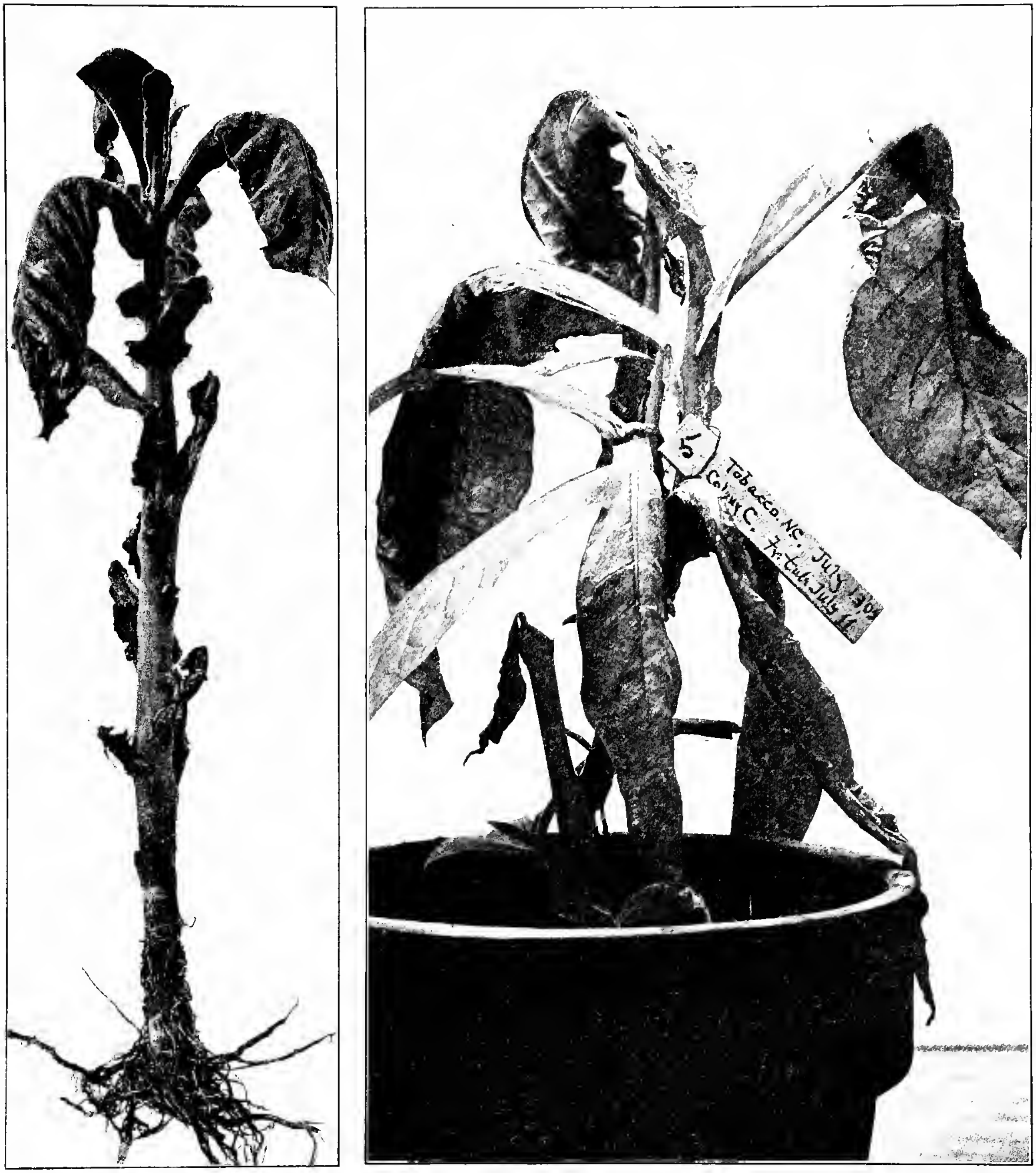
PLATE 26.



Effect of Bacterium solanacearum on a tomato-plant.

The left-hand plant was inoculated in the stem on July 5, by means of a few needle-pricks. The right-hand plant was pricked with a sterile needle. The infectious material was a subculture from a poured-plate colony. This was obtained from the interior of a potato-stem, naturally infected, in District of Columbia. Photographed July 11, 1904. About one-third natural size.

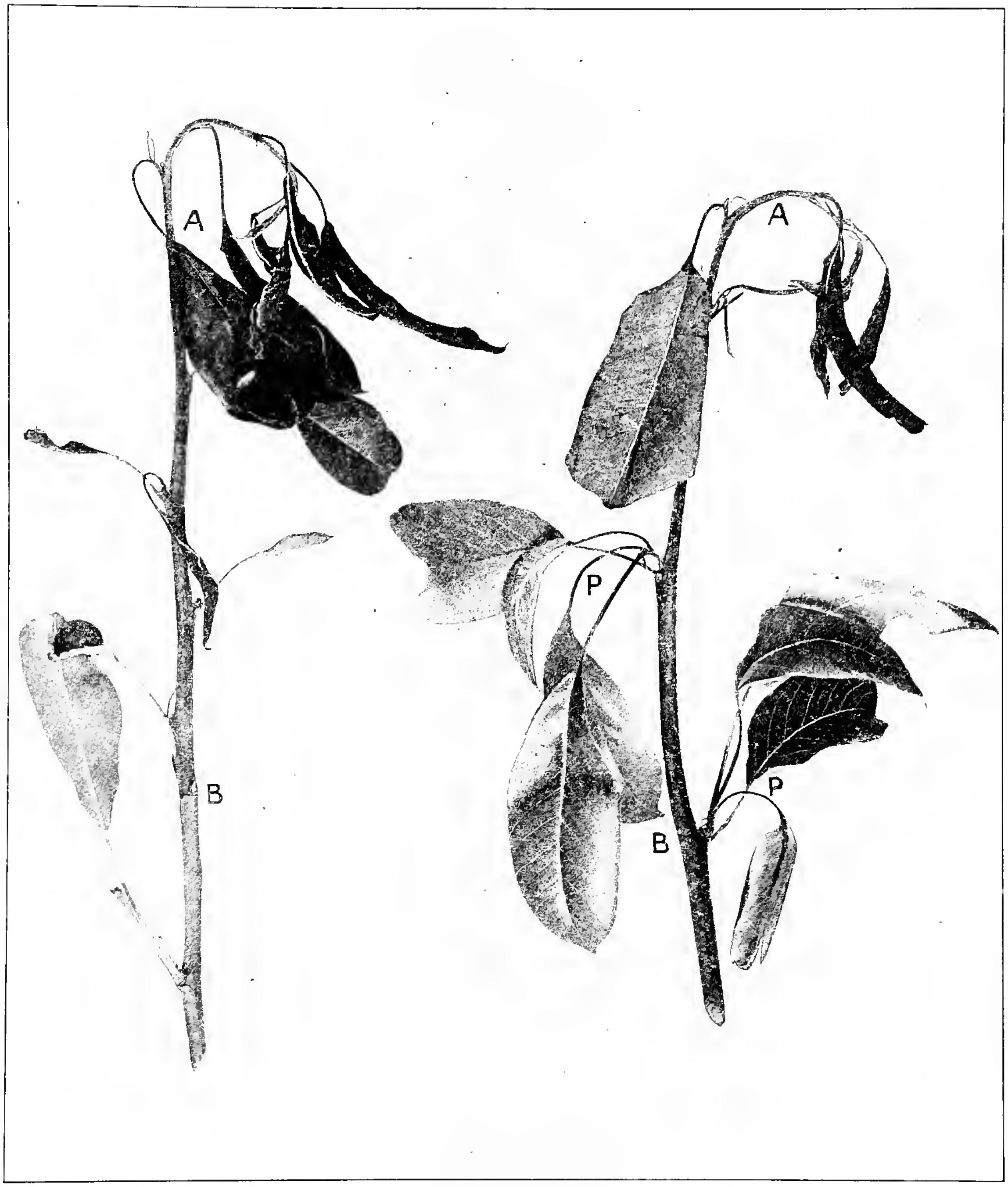
PLATE 27.



The Granville tobacco-wilt, a bacterial disease.

The left-hand plant wilted naturally in the field, was pruned, transplanted to the hothouse, recovered for a few weeks, developed the leaves shown, and then wilted again. Photographed June 28; one-third natural size.

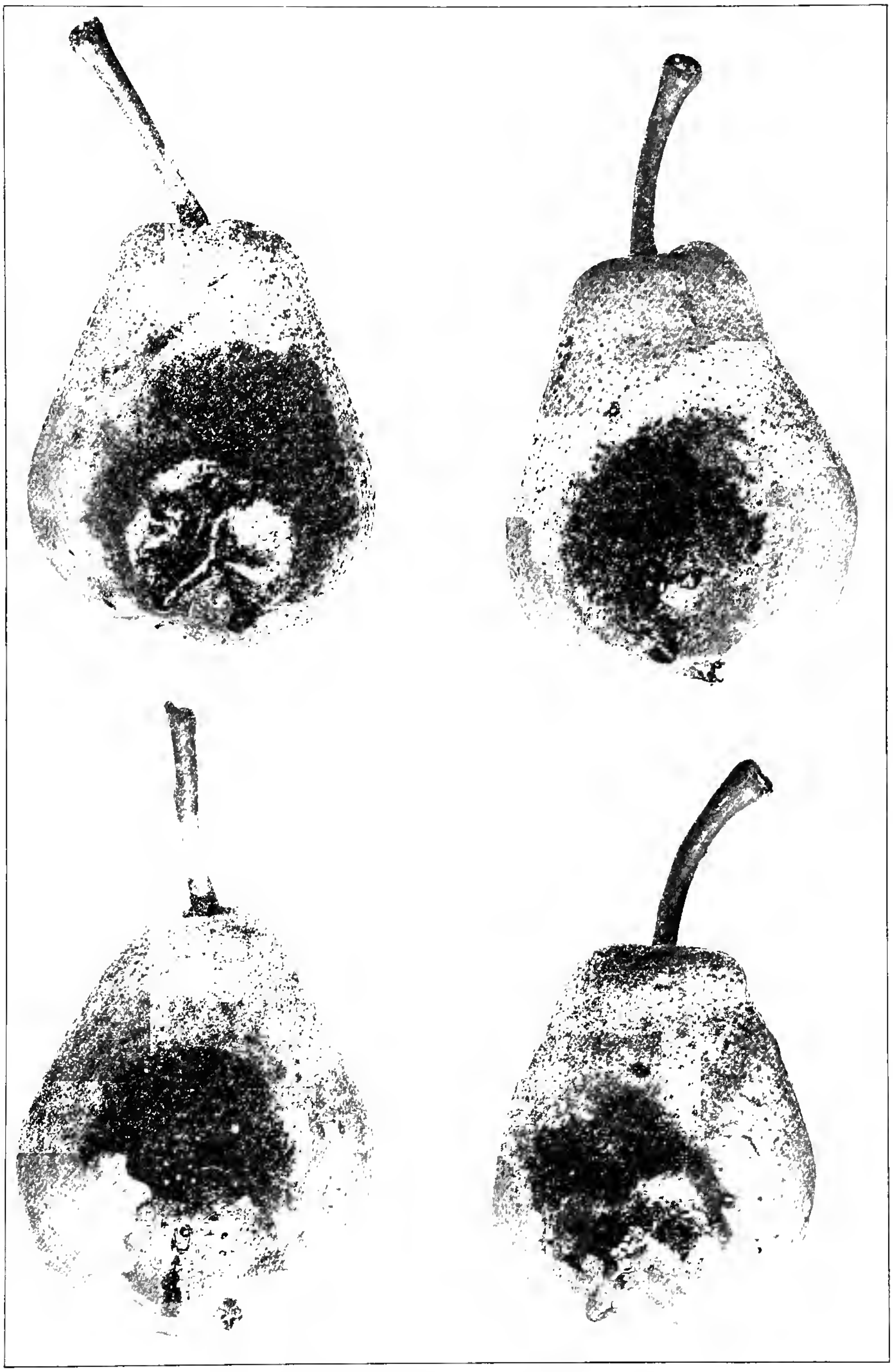
In this disease the vascular ring is browned and cavities are formed in the stem. The bacterial slime is gray-white and abundant. Fungi were not present in the plants examined by me. Poured plates were made from the interior of several such stems, and these yielded practically pure cultures of one organism. A subculture from one of these colonies was used to inoculate the right-hand plant. The needle-pricks were made on July 13, 1905. Photographed August 3; one-half natural size. Twelve plants were inoculated at this time and all contracted the disease. The signs and lesions were the same as in the plants obtained from the field. The organism causing this disease is closely related to Bact. solanacearum.



Pear-shoots blighted by Bacillus amylovorus.

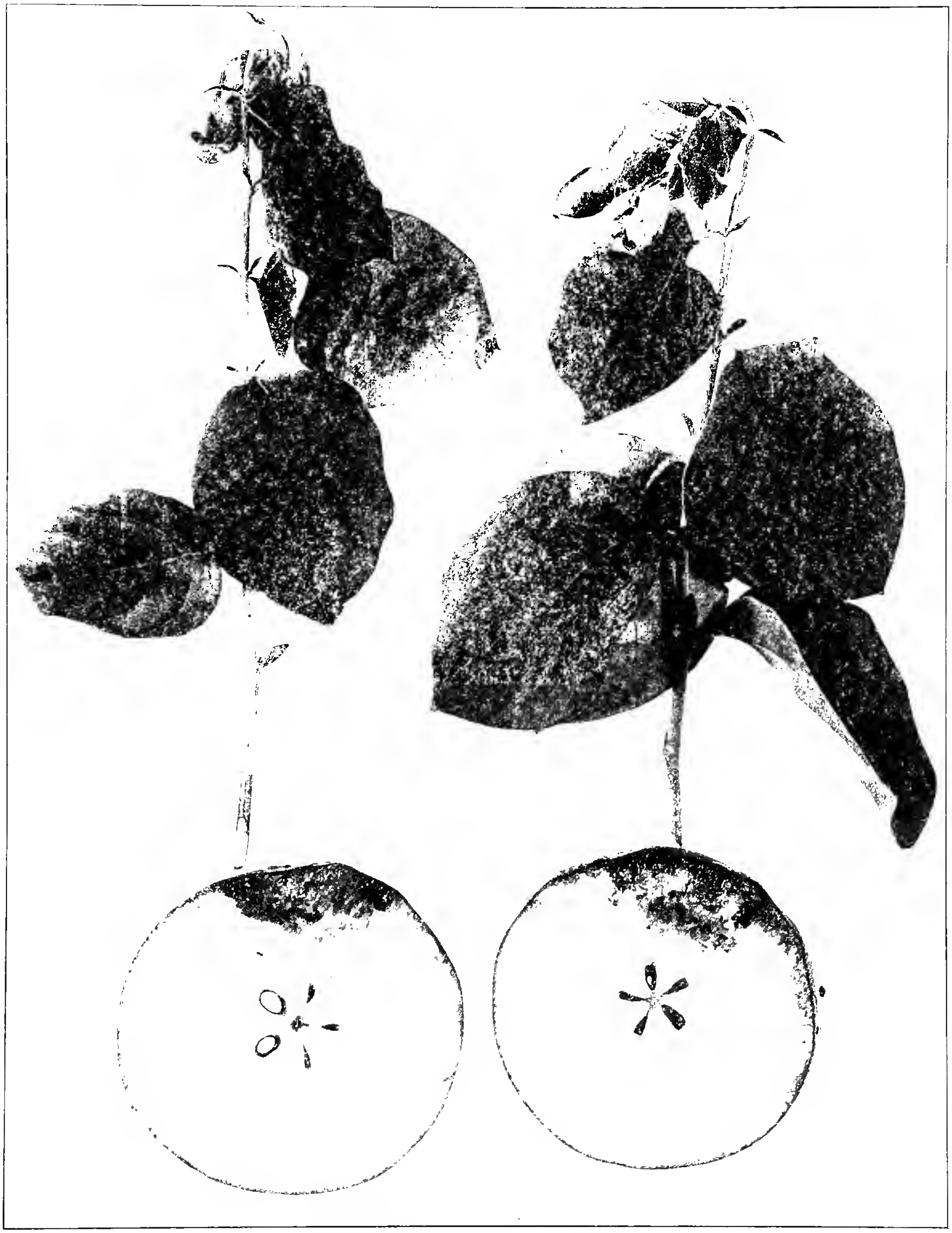
Inoculations by needle-pricks on rapidly growing stems. The infectious material came from a green apple. The poured plates yielded practically pure cultures and the inoculations were made directly from colonies in these plates. Of 12 inoculations (8 shoots, 4 fruits) 11 were successful. Needle-pricks made July 10, 1905. Photographed July 20. The lower leaves were still green; the tops (A and A) had shriveled and browned; the bacteria had passed downward in the bark to points below B and B, and they had also run out into the petioles (P and P) and had browned them, but the blades of these leaves were still green.

PLATE 29.



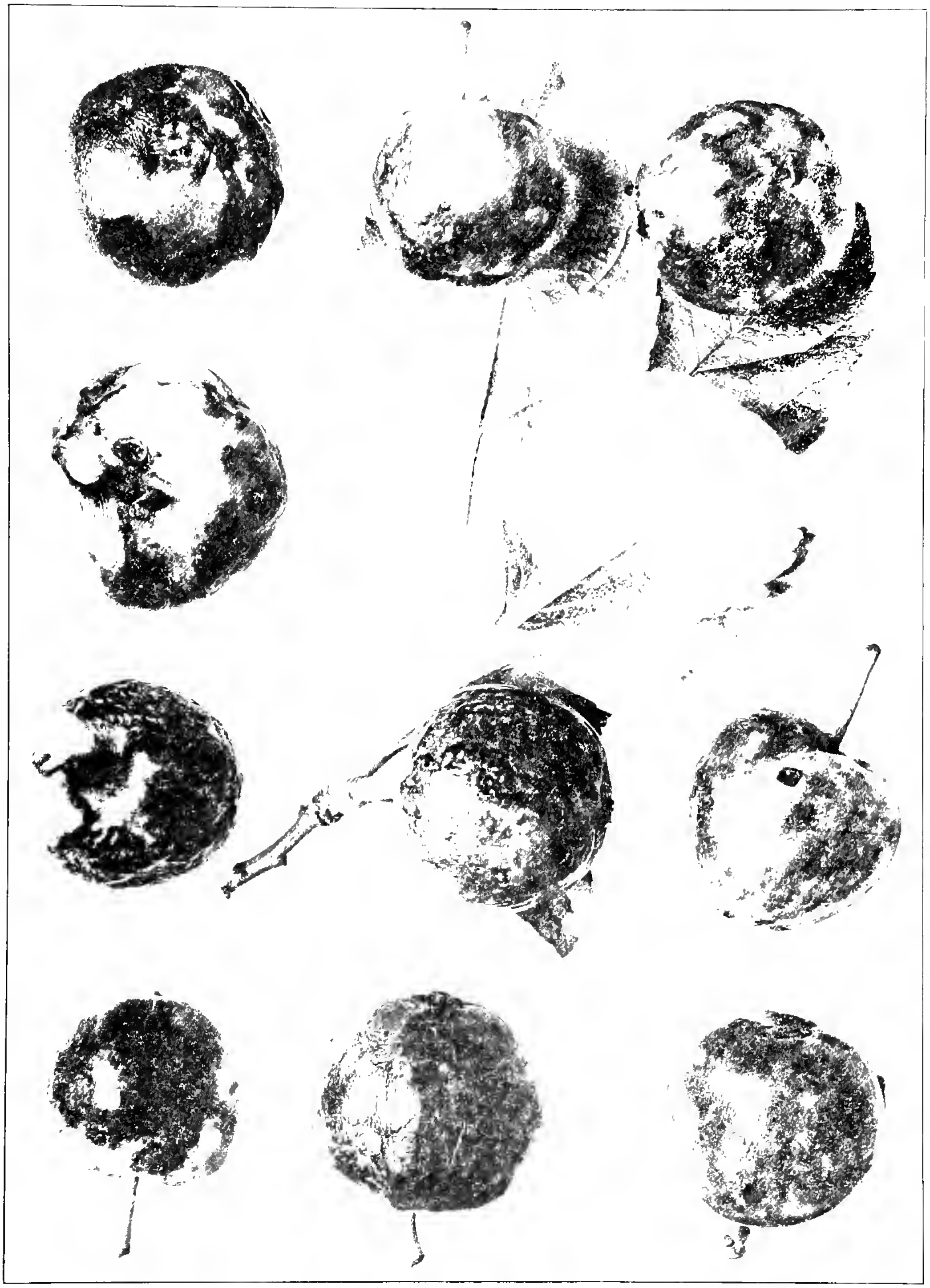
Green pears inoculated with Bacillus amylovorus.

These were inoculated from the same poured plate and at the same time as the shoots shown in plate 28, but the photographs were made two days earlier. The inoculations were by means of a few needle-punctures. The inoculated parts browned, softened, shriveled slightly, and were extruding bacterial slime from small cracks and from many stomata. Beyond the browned area there was a water-soaked area. The internal injury was extensive—in one fruit nearly the whole interior had softened and was occupied by the bacteria. These tissues were filled with grayish bacterial slime to such an enormous extent that on handling them the fingers dripped with it. Inoculated July 10, 1905. Photographed July 18; about natural size. Poured plates made from the interior of these fruits yielded pure cultures of B. amylovorus.



Blighted quince-shoots and pear-fruits.

Twelve inoculations were made and all were successful—8 quince-shoots and 4 pear-fruits. None of the checks contracted the disease. The organism was introduced by delicate needle-pricks. The subculture used was derived from a poured-plate colony obtained from the interior of one of the pears shown in plate 29. Owing, no doubt, to the riper and therefore less susceptible condition of the tissues, and possibly also to somewhat cooler weather, the disintegration of the deeper tissues of the fruits had not proceeded as far as in the pears inoculated on the same tree 10 days earlier; in those fruits first inoculated the decay was from 5 to 10 times as extensive on the 8th day as in the second inoculations on the 11th day. Inoculated July 20, 1905. Photographed July 31



Small green apples blighted by Bacillus amylovorus.

One of these fruits furnished the bacteria used to inoculate the shoots and fruits shown in plates 28 and 29. The interior of these fruits was gorged with a gray-white bacterial slime. The surface was browned and shriveling in many places and water-soaked in others. Bacteria were oozing from the darker parts. Every one of many fruits examined had been wounded by the curculio, and probably infection occurred in this manner. Arlington Farm, Virginia. Photographed June 30, 1905.

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('90). Kramer, Ernst. Die Bakteriologie in ihren Beziehungen zur Landwirtschaft und den landwirtschaftlich - technischen Gewerben. Theil I. Die in der Landwirtschaft durch Bakterien bewirkten Vorgänge. Mit 36 Abbild. Wien, Verlag von Karl Gerolds Sohn, 1890. 8vo. 171 pp. II Theil. Die Bakterien in ihren Verhältnisse zu den landwirtschaftlichen-technischen Gewerben. 8vo. pp. VI, 178, with 79 figures. Vienna, Gerolds Sohn, 1891.

('91). Eisenberg, James. Bakteriologische Diagnostik. 3d ed. pp. XXXI, 509. Hamburg and Leipsic, Leopold Voss, 1891.

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('91). Woodhead, German Sims. Bacteria and their products. London, Walter Scott, Ltd., 1891. 12mo. pp. XIII, 459, with 20 photomicrographs.

('92). Sternberg, Geo. M. Manual of Bacteriology. pp. XII, 886. 8 plates and 268 figures in the text. 8vo. New York, Wm. Wood & Co., 1892.

('94). Frankland, Percy, and Frankland, Mrs. G. C. Micro-organisms in water. London, Longmans, Green & Co. pp. XI, 532, with figures, 1894.

('95). Abbott, A. C. The principles of bacteriology. A practical manual for students and physicians. 3d ed. pp. XII, 493, with 98 figures, 17 of which are colored. Lea Brothers & Co., Philadelphia, 1895. Last (6th) ed. in 1902, pp. XI, 641.

('95). Itzerott und Niemann. Mikrophotographischer Atlas der Bakterienkunde. Mit 126 mikrophotographischen Abbildungen in Lichtdruck auf 21 Tafeln. Leipzig, Johann Ambrosius Barth (Arthur Meiner), 1895.

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For criticisms on that portion of this book which relates to plant diseases, see Centralb. f. Bakt., 2 Abt. 1899 p. 271, 800, and 1901, 88, 128, 196. English translation in 1900 by A. Coppen-Jones, under title, The structure and functions of bacteria. 2 German ed. in 1903.

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('98). Duclaux, E. Traité de Microbiologie. T. I, Microbiologie général. Paris, Masson et Cie. 1898. pp. III, 632, 61 text figures.

('98). Procedures recommended for the study of bacteria, with especial reference to greater uniformity in the description and differentiation of species. Being a report of a committee of American bacteriologists to the Committee on the Pollution of Water Supplies of the American Public Health Association. (Submitted at the meeting of the association in Philadelphia, Sept., 1897.) Concord, N. H., The Rumford Press, 1898. pp. 47, with charts.

This pamphlet should be in every laboratory, and in the hands of every student.

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('02). Eyre, J. W. H. The elements of bacteriological technique, a laboratory guide for the medical, dental, and technical student. 170 illustrations. Philadelphia and London, W. B. Saunders & Co., 1902. pp 371.

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Cette collection touche comme principaux sujets; charbon, rouget, choléra des poules, pneumonie, lèpre, suppuration, peste, gonocoque, choléra, fièvre typhoide, morve, tuberculose, lèpre, actinomycose, dipthérie, tétanos, etc., et les maladies à protozoaires. coccidies, paludisme, maladie de la mouche tsé-tsé, trypanosomes, etc. Conditions de la publication. La collection comprend actuellement 65 planches du format 80 x 62 centimètres, tirées en couleurs sur papier toile très fort, munies d'oeillets permettant de les suspendre sur deux pitons et réunies, dans un carton disposé spécialement à cet effet. Elle est accompagnée d'un texte explicatif rédigé en trois langues (français, allemand, anglais) Prix: 250 francs (port en sus). Les planches ne sont pas vendues separement.

('—). Park, William H. Bacteriology in Medicine and Surgery. Lea Brothers & Co., New York. 12mo., 688 pages, with 87 illustrations in black and colors, and 2 colored plates.

('02). Roger, G. H. Les maladies infectieuses. Paris, Masson et Cie, 1902, T. i, pp. xiv, 664; T. ii, pp. 665-1,520; av. 117 fig.

('02). Matzuschita, Teïsi. Bacteriologische Diagnostik. Zum Gebrauche in den bacteriologischen Laboratorien und zum Selbstunterrichte. Für Aerzte, Tierärzte und Botaniker. Jena (G. Fischer), 1902, pp. xvii, 692, mit 1 Taf.

('02). Hewlett, Richard T. A Manual of Bacteriology, Clinical and Applied, 2d Edition. London (Churchill), 1902, p. 533, with 20 plates.

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('03). Williams, Herbert U. A manual of bacteriology. Ninety-nine illustrations. 3d ed., revised and enlarged. Philadelphia, P. Blakiston's Son & Co., 1903. pp. xv, 351.

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('83). Molisch, Hans. Ueber d. Mikrochem. Nachw. v. Nitraten u. Nitriten in d. Pflanze mittelst Diphenylamin oder Brucin. Berichte der Deutschen Botanischen Gesellschaft, 1883. pp. 150-155. See also Bot. Centralb., Bd. xiv, 1883, pp. 355-356.

('84). Gautier, Armand. Traité de chimie appliquée à la physiologie et à l'hygiene. Paris, 1884.

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This author gives a method of estimating the quantity of acid in plants when not free, i. e., when united with bases.

('84). Moll, J. W. Eene nieuwe mikrochemische looizuurreactie. Maandblad voor Natuurwetenschappen, 1884. Rev. in Bot. Centralb. Bd. xxiv, p. 250.

Best method (micro-chemical) of detecting tannin in cells. He soaks small pieces of tissue in saturated solution (7 per cent) acetate of copper 8-10 days, and longer will not injure. Then they are sectioned and treated for a *few* minutes with a drop of 0.5 per cent sol. iron acetate. Too long an exposure browns the cell walls. The sections are then washed in water, transferred to alcohol and finally mounted in glycerine or glycerine jelly. If the test cannot be made at once, the specimens will not be injured by preserving in alcohol, after the soaking in acetate of copper. The tannin-bearing cells become green or blue.

('85). Hanausek, T. F., and Pammer, L. Ueber die Löslichkeitsverhältnisse des Kautschuks. Zeitschrift des allg.- österr. Apotheker-Vereins, 1885, No. 31, pp. 486-488, with a table. Rev. in Bot. Centralb., Bd. xxv, 1886, p. 308.

Note on best solvents for rubber.

('85). Strasburger, Ed. Zur mikroskopischen Technik. Bot Centralb., Bd. xxiv, 1885, pp. 150-157

According to Strasburger, Faber's yellow pencils are the best for writing on glass or porcelain. To find particular places in a preparation, he makes rings on the stage of the microscope to each side of the opening, and, when the desired field is in view, corresponding ones on the slide. The best method of making eau de Javelle is said to be the following:

(*a*) Take 20 parts of 25 per cent chloride of lime (Chlorkalkes), stir up in 100 parts of water, and let stand for a time

(*b*) Dissolve 15 parts of pure potash in 100 parts of water.

Add *b* to *a*, and, after one to several days, filter and use the filtrate. If lime still remains in the fluid, add a few drops of potash solution and filter out precipitate.

('85). Lehmann, O. Physikalische Technik. Specielle Anleitung zur Selbstanfertigung physikalischer Apparate. pp. xii, 416, with 882 wood cuts and 17 tables. Leipsic, Wm. Engelmann, 1885.

('85). Noll, F. Eau de Javelle, ein Aufhellungs- und Lösungsmittel für Plasma. Botan. Centralb., Bd. xxi, 1885, pp. 377-380.

Author tells how to make and how to use eau de Javelle. Alcoholic material is best suited for treatment. Fresh protoplasm does not dissolve completely, but leaves detritus and granules. Strasburger, Ibid., Band xxiv, p. 157, says this is not eau de Javelle.

('85). Kraus, C. Ueber amphotere Reaction der Pflanzen-Säfte. Botan. Centralb., Bd. xxiv, 1885, p. 287.

A. Meyer summarizes some of Kraus' results obtained from the pith parenchyma of twenty plants. Kraus claims that neutral litmus would not answer. He used red and blue litmus Little pieces were thrown into the sap and left a long time. He got both reactions.

('86). Pfeffer, W. Ueber Aufnahme von Anilinfarben in lebende Zellen. Untersuchungen a. d. Bot. Inst. zu Tuebingen, Leipzig, 1886, Bd. ii, Heft 2, pp. 179-329.

('86). Bachmann, E. Spektroscopische Untersuchugen von Pilzfarbstoffen. Plauen, 1886.

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('90). Curtman, Charles O. Chemical reagents and the spectroscope. 12mo. pp. 256, with 12 plates. John L. Boland Book and Stationery Co., St. Louis, 1890.

A useful little book.

('90). Nickel, Emil. Die Farbenreactionen der Kohlenstoffverbindungen. 2d ed. pp. viii, 134. Berlin, Verlag von Hermann Peters, 1890.

('91-'92). Ostwald, Wilhelm. Lehrbuch der allgemeinen Chemie. Zweite gänzlich umgearbeitete Auflage. Leipzig, 1891-1892. 2 Bd.

The fourth book of the second edition was translated into English by M. M. Pattison Muir, under the title "Solutions." London, 1891, pp. xiii, 316, 8 vo.

('92). Trimble, Henry. The tannins. pp. 168. J. B. Lippincott Co., Philadelphia, 1892.

('92). Zimmermann, A. Die botanische Mikrotechnik. Ein Handbuch der Mikroskopischen Präparations- Reaktions- und Tinktionsmethoden. Mit 63 Abbild. im Text. Tübingen, 1892. pp. x, 278. English translation by James Ellis Humphrey. Henry Holt & Co., New York, 1893.

('93). Lea, A. Sheridan. The chemical basis of the animal body. New York, Macmillan & Co., 1893. pp. 288. Forms Part v of 6th ed. of Foster's larger Physiology.

('93). Bender, Adolf, und Erdmann, Hugo. Chemische Präparatenkunde. 2 vols. Bd. i. Anleitung zur Darstellung anorganischer Präparate, von Bender. pp. viii, 530, with 102 figures, 1893. Bd. ii. Anleitung zur Darstellung organischer Präparate, von Erdman. pp. xii, 610, with 41 figures. Stuttgart, Ferdinand Enke, 1894.

('93-'95). Schmidt, Ernst. Ausführliches Lehrbuch der Pharmaceutischen Chemie. Braunschweig, Fr. Vieweg & Sohn. 3d ed. Inorg., 1893. Organic, 1895. pp. 648, vi. 4th ed., 1901. pp. xxxiii, 1,944.

('94). Behrens, H. A manual of microchemical analysis. With an introductory chapter by John W. Judd. With 87 illustrations. pp. xxv, 246. London and New York, Macmillan & Co., 1894.

('94). Landolt und Boernstein. Physikalisch-chemische Tabellen. 2d ed. pp. xi, 563. Berlin, Julius Springer, 1894.

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('94). Schulz, G., and Julius, P. Systematic survey of the organic colouring matters. Translated from the German and edited with extensive additions by Arthur G. Green. pp. viii, 205. London and New York, Macmillan & Co., 1894.

Very useful.

('94). Lidforss, B. Ueber die Wirkungssphäre der Glycose u. Gerbstoff-Reagenten. Sep. Abdr. aus Lunds Univ. Arsskr. T. xxviii, 14 pp. Rev. Bot. Centralb., 1894. Bd. lix, p. 281.

Author says tannins give most of the protein reactions, and may thus lead investigators to many wrong conclusions. The following test is given for grape sugar in plant tissues: The parts to be tested are soaked for some time in an alcoholic copper solution, and then brought to a boil over a water bath. This causes a precipitate of copper oxide (oxydul) in all of the cells which contained grape sugar. The alcoholic copper solution is made as follows: Add some acetic acid and glycerin to an alcoholic solution of copper acetate This is then mixed with an equal volume of alcoholic solution of caustic soda (Natronlösung). This mixture is as sensitive as Fehling's solution, and is not reduced by various substances which reduce Fehling. A great number of soluble reducing substances are removed by the alcohol from the plant cells, while the sugar remains behind.

Barfoed's reagent consists of acetate of copper dissolved in water and made acid by acetic acid. It is useful for detection of sugar in some cases where Fehling's solution is worthless, i. e., in the presence of Phloroglucin, Aesculin, Quercit, which reduce the latter. The former, on the contrary, is reduced by Hydrochinon and Resorcin.

('95). Cross and Bevan. Cellulose. An outline of the chemistry of the structural elements of plants, with reference to their natural history and industrial uses. pp. vi, 320, with 14 plates. London, Longmans, Green & Co., 1895.

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('95). von Lippman, E. O. Die Chemie der Zuckerarten. 8vo. Second edition. pp. xxvi, 1,174. Braunschweig, Fr. Vieweg u. Sohn, 1895.

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('96). Merck's Index. An encyclopædia for the physician and the pharmacist; stating the names and synonyms; source or origin; chemical nature and formulas; physical form, appearance, and properties; melting and boiling points; solubilities; gravities and percentage strengths; physiological effects; therapeutic uses; modes of administration and application; regular and maximum dosage; incompatibles; antidotes; special cautions; hints on keeping and handling; methods of testing; market values, etc., of the chemicals and drugs used in medicine, in chemistry, and in the arts. 2d ed. Merck & Co., New York, 1896.

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('96). Lefevre, Léon. Traité des matières colorantes organiques artificielles, de leur préparation industrielle et de leur applications. 2 vols. 8vo. pp. xx, 1,648, with 31 text figures and 261 dyed or printed samples of silk, wool, cotton, paper, and leather, prepared under the direction of the author especially for this edition. Paris, G. Masson, 1896.

('97). Davenport, C. B. Experimental morphology Part 1. Effect of chemical and physical agents upon protoplasm, 1897. The Macmillan Co., New York. pp. xiv, 280, 74 figs.

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('97). Bausch, Edward. Manipulation of the microscope. Bausch and Lomb Optical Co., Rochester, N. Y. 3d ed. 200 pp. 1897.

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('98). Schroeder von der Kolk, J. L. C. Kurze Anleitung zur mikroscopischen Krystalbestimmung. pp. 60, with text figures. Wiesbaden, C. W. Kreidel's Verlag, 1898.

('98-'03). ALLEN, ALFRED H. Commercial organic analysis. A treatise on the modes of assaying the various organic chemicals and products employed in the arts, manufactures, medicine, etc., with concise methods for the detection of impurities, adulterations, etc. 8vo.

Vol. I. Alcohols, neutral alcoholic derivatives, etc., ethers, vegetable acids, starch, sugars, etc. 3d ed., 1898, pp. xii, 557.

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Vol. II, Part II. Hydrocarbons, mineral oils, lubricants, benzenes, naphthalenes and derivatives, creosotes, phenols, etc. 3d ed., 1901, pp. viii, 330.

Vol. II, Part III. Terpenes, essential oils, resins, camphors, etc. 3d ed. preparing.

Vol. III, Part I. Tannins, dyes, and coloring matters. 3d ed., enlarged and rewritten. Illustrated. 1900, pp. xvi, 589.

Vol. III, Part II. The amines, hydrazines and derivatives, pyridine bases, the antipyretics, etc. Vegetable alkaloids, tea, coffee, cocoa, etc. 8 vo. 2d ed., 1892, pp. viii, 584.

Vol. III, Part III. Vegetable alkaloids, non-basic vegetable bitter principles. Animal bases, animal acids, cyanogen compounds, etc. 2d ed., 8 vo., 1896, pp. xii, 508.

Vol. IV. The proteids and albuminous principles. 2d ed., 1898, pp. xi, 584.

('99). COHN, ALFRED I. Indicators and test papers, their source, preparation, application, and tests for sensitiveness, etc. New York, John Wiley & Sons; London, Chapman & Hall, Ltd., 1899. pp. IX, 240.

Very useful.

('99). The Dispensatory of the United States of America. 18th ed., 1899. Philadelphia, J. B. Lippincott Company, pp. XLV, 1,909.

('99). BEHRENS, H. Anleitung zur mikrochemischen Analyse. pp. XI, 242, with 96 figures 2d ed. Hamburg and Leipsic, Voss, 1899.

('00). SCHOENICHEN, WALTER, UND KALBERLAH, ALFRED. B. Eyferth's Einfachste Lebensformen des Tier- und Pflanzenreiches. Naturgeschichte der mikroskopischen Süsswasserbewohner. 3 vollständig neubearbeitete und vermehrte Aufl. Mit über 700 Abbildungen auf 16 Tafeln in Lichtdruck nach Zeichnungen von Dr. A. Kalberlah. Braunschweig, Verlag von Benno Goeritz, 1900. pp. VIII, 550.

('00). SUTTON, FRANCIS. A systematic handbook of volumetric analysis. 8th ed., enlarged and improved, 1900. pp. XI, 640, with figures. London, J. and A. Churchill.

This book should be in every laboratory.

('00). VAN RIJN, J. J. L. Die Glykoside chemische Monographie der Pflanzenglykoside, nebst systematischer Darstellung der künstlichen Glykoside. Gebrüder Borntraeger, Berlin, 1900. pp. XVI, 511.

('00). BEHRENS, H. Mikrochemische Technik. Hamburg u. Leipzig, Leopold Voss, 1900. pp. VIII, 68.

('00). BUECHLER, MAX. Die Diastasen, 1900.

Not seen

('01). GAGE, S. H. The Microscope. An introduction to microscopic methods and to histology. 8th ed., revised, 1901. pp. IV, 299, with over two hundred figures. Comstock Publishing Co., Ithaca, N. Y.

Deserves a place in every laboratory

('01). COBLENTZ, VIRGIL. A manual of volumetric analysis, treating on the subjects of indicators, test-papers, alkalimetry, acidimetry, analysis by oxidation and reduction, iodometry, assay processes for drugs with the titrimetric estimation of alkaloids, estimation of phenol, sugar, tables of atomic and molecular weights. P. Blakiston's Son & Co., Philadelphia, 1901. pp. VIII, 181.

('01). LEE, ARTHUR BOLLES. The Microtomist's Vade-Mecum, a handbook of the methods of microscopic anatomy. 5th rev. ed. Philadelphia, P. Blakiston's Son & Co., 1901. pp. XIV, 532.

Very useful. French ed by Lee and Henneguy. Paris, Octave Doin, 1902.

('01). CROSS, C. F., AND BEVAN, E. J. Researches on cellulose, 1895-1900. Longmans, Green & Co., London, New York, and Bombay, 1901. pp. VII, 180.

('01). HANAUSEK, T. F. Lehrbuch der technischen Mikroskopie. With 256 text figures. Stuttgart, Ferdinand Enke, 1901. pp. X, 456.

('01). RAWSON, CHRISTOPHER, GARDINER, WALTER M., AND LAYCOCK, W. F. A dictionary of dyes, mordants, and other compounds used in dyeing and calico printing. London, Chas. Griffin & Co., Ltd.; Philadelphia, J. B. Lippincott Co., 1901. pp. 372.

('02). OMÉLIANSKI, W. Ueber die Gärung der cellulose. Centralb. f. Bakt., 2 Abt., Bd. VIII, Nos. 7-13, pp. 193-201, 225-231, 257-263, 289-294, 321-326, 353-361, 385-391, 1 text fig. and 1 plate. Jena, 1902.

Oméliansski finds two morphologically similar bacteria capable of fermenting pure cellulose (Swedish filter paper) in mineral solutions with chalk. Both grow anaerobically, and bear spores in a swollen terminal part. One called the hydrogen ferment breaks up cellulose with the formation of hydrogen, carbon dioxide, acetic acid, and butyric acid. The other, called the marsh-gas ferment, breaks up cellulose with the formation of marsh-gas, carbon dioxide, acetic acid, and butyric acid. The cultures were made by the selective method, by which means most of the accompanying forms were crowded out. The hydrogen ferment was isolated from the methane ferment by heating the material used for the first transfer (from the methane-yielding ferment) for 15 minutes at 70°, the subsequently inoculated flasks then gave only the hydrogen fermentation. The organism of the latter was isolated pure on potato, but only after many trials and with feeble growth and weak ferment powers. The methane bacterium was not obtained pure in colonies. Neither organism colored blue with iodine. The experiments were begun in 1894 and carried through a long series of years, involving an enormous amount of painstaking labor.

('02). MANN, GUSTAV. Physiological histology. Methods and theory. pp. XV, 488. Oxford, The Clarendon Press, 1902.

('02). HOEBER, RUDOLF. Physikalische chemie der Zelle und der Gewebe. Wilhelm Engelmann, Leipzig, 1902, pp. XII, 344, with 21 fig.

('03). KUESTER, ERNST. Pathologische Pflanzenanatomie. Verlag von Gustav Fischer, Jena, 1903, pp. VII, 312, with 121 figures.

('03). DAVENPORT, CHARLES BENEDICT. Experimental Morphology. Part II. Effects of Chemical and Physical Agents upon Growth. 8vo. The Macmillan Co., New York.

('03). Peirce, George James. A Text-Book of Plant Physiology. Henry Holt & Co., New York, 1903, pp. vi, 291.

('03). Cohn, Alfred I. Tests and reagents, chemical and microscopical, known by their author's names, together with an index of subjects. 1st ed. 383 pp. New York, John Wiley & Sons; London, Chapman & Hall, Ltd., 1903.

('03). von Fuerth, Otto. Vergleichende chemische Physiologie der niederen Tiere. Jena, Gustav Fischer, 1903. pp. xiv, 670.

('04). Nuttall, George H. F. Blood immunity and blood relationship; a demonstration of certion blood-relationships amongst animals by means of the precipitin test for blood. pp. xii, 444. Cambridge (England) University Press, 1904.

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V. Books and Papers of More or Less General Interest.

('28). Ehrenberg, C. G. Symbolæ physicæ, seu icones et descriptiones animalium evertebratorum sepositis insectis quæ ex itinere per Africam Borealem et Asiam Occidentalem Friderici Guilelmi Hemprich et Christiani Godofredi Ehrenberg medicinæ et chirurgiæ doctorum studio novæ aut illustratæ redierunt percensuit et regis iussu et impensis edidit Dr. C. G. Ehrenberg. Decas prima. Berolini ex Officina Academica Venditur a mittlero mdcccxxviii.

('30). Ehrenberg, C. G. Beiträge zur Kenntnis der Organisation der Infusorien, etc. Printed 13 Aug., 1830, as part of Abhandl. d. K. Akad. d. Wissensch. zu Berlin. Physic. Klasse for the year 1830. As a whole the volume bears date of 1832.

('32). Ehrenberg, C. G. Die geographische Verbreitung der Infusionsthierchen in Nord-Afrika und West-Asien, etc. Abhandl. d. K. Akad. d. Wissensch. zu Berlin. Physik. Klasse. For the year 1829. Printed 1832.

('32). Ehrenberg, C. G. Ueber die Entwickelung und Lebensdauer der Infusionsthiere, etc. Abhandl. d. K. Akad. d. Wissensch. zu Berlin, 1831. Printed January, 1832.

('38). Ehrenberg, C. G. Die Infusionsthierchen als vollkommene Organismen. Ein Blick in das tiefere organische Leben der Natur, von Christian Gottfried Ehrenberg zu Berlin. Nebst einem Atlas von vierundsechszig colorirten Kupfertafeln gezeichnet vom Verfasser. Leipzig, Verlag von Leopold Voss, 1838. pp. 548, folio.

('40). Henle, J. Pathologische Untersuchungen. Berlin, August Hirschwald. pp. vi, 274. 1840.

('54). Schroeder, H., und von Dusch, Th. Ueber Filtration der Luft in Beziehung auf Fäulniss und Gährung. Annalen der Chemie und Pharmacie, Bd. lxxxix (Neue Reihe, Bd. xiii), Heft 1. pp. 232-243, Heidelberg, 1854.

Extremely interesting historically. These authors were the first to show that boiled beef bouillon and similar easily putrescible fluids could be preserved indefinitely if protected from the floating matter of the air by means of cotton plugs. Boiled milk, on the contrary, decayed as certainly under the cotton as when exposed directly to the air, for reasons unknown to them. The exact nature of this floating matter of the air was not determined. That remained for Pasteur to work out some years later by means of many ingenious experiments.

('54). Cohn, Ferdinand. Untersuchungen über die Entwicklungsgeschichte der mikroscopischen Algen und Pilze. Der Akademie übergeben den 1 Mai, 1853. Nov. Act. Acad. Cæs. Leop.-Carol. Naturæ Curiosorum. Vol. xxiv, Pars 1, 1854. pp. 103-256, 6 plates. Breslau and Bonn.

('59). Schroeder, H. Ueber Filtration der Luft in Beziehung auf Fäulniss, Gährung und Krystallisation. Annalen der Chemie und Pharmacie, Bd. cix (Neue Reihe Bd. xxxiii), Hft. 1, pp. 35-52, 1859. Leipzig und Heidelberg.

('60). Pasteur, Louis.. Expériences relatives aux générations dites spontanées. C. R. des sé. de l'Acad. des Sci., Paris, 1860. pp. 303-307.

('62). Pasteur, Louis. Mémoire sur les corpuscles organisés qui existent dans l'atmosphère. Examen de la doctrine des générations spontanées. Ann. de Chimie et Physique, 1862. 3 série, T. lxiv, pp. 5-110.

('65). Davaine, C. Sur l'existence et la recherche des bactéridies dans la pustule maligne. C. R. d. sé. et mém. de la société de biol. for 1864, p. 93-94 (comptes rendus part). Paris, 1865.

('68). Roberts, W. Chandler. On the occurrence of organic appearances in colloid silica obtained by dialysis. Journ. of the Chem. Soc. of London. New series, vol. vi, 1868, pp. 274-276, 2 figs. of fungi.

Roberts appears to have been the first to observe the growth of organisms on silicate jelly.

('72). Cohn, F. Untersuchungen über Bacterien. Cohn's Beitr. zur Biol. der Pflanzen, Bd. 1. 2 Hft. 1872. pp. 127-224. 1 plate.

('73). Lister, Joseph. A further contribution to the natural history of Bacteria and the germ theory of fermentative changes. Quarterly Journal of Microscopical Science. 1873. Vol. xiii. pp. 380-408.

('73). Pasteur, Louis. Etudes sur le vin, ses maladies, causes qui les provoquent, procédés nouveaux pour le conserver et pour le vieillir. Deuxième édition revue et augmentée. Avec 32 planches imprimées en couleur et 25 gravures dans le texte. Paris, 1873. F. Savy. pp. iv, 344.

('76). Pasteur, Louis. Études sur la bière, ses maladies, causes qui les provoquent, procédé pour la rendre inaltérable, avec une théorie nouvelle de la fermentation. 12 plates, 85 text figures. Paris, 1876. Gauthier-Villars. pp. viii, 387.

('77). von Naegeli, C. Die niederen Pilze in ihren Beziehungen zu den Infectionskrankheiten und der Gesundheitspflege. München. R. Oldenbourg, 1877. pp. xxxii, 285.

('78). Lister, Joseph. On the lactic fermentation and its bearing on pathology. Transactions of the Pathological Society of London. 1878. Vol. xxix, pp. 425-467.

('79). Nencki, M., u. Giacoso, P. Giebt es Bacterien oder deren Keime in den Organen gesunder lebender Thiere? Journ. f. Prakt. Chem., 1879. Bd. xx, p. 34-44.

('82). Mott, F. W., and Horsley, V. H. On the existence of bacteria, or their antecedents, in healthy tissues. Journ. of Physiol, vol. iii, 1880-1882. pp. 188-194.

('82). Engelmann, Th. W. Zur Biologie der Schizomyceten. Bot. Zeitung, 1882, col. 321-325 and 337-341.

('84). Metschnikoff, E. Ueber die Beziehung der Phagocyten zu Milzbrandbacillen. Archiv f. patholog. Anat. u. Physiologie u. f. Klin. Med., Bd. LXXIX, 1884. pp. 502-526. 2 plates.

('86). Bolton, Meade. Ueber das Verhalten verschiedener Bacterienarten im Trinkwasser. Ztschr. f. Hyg. Bd I, 1886. pp. 76-114.

('86). Abbe, E. Ueber Verbesserungen des Mikroskops mit Hilfe neuer Arten optischen Glases. Sitzungsber. der medicin.-naturw. Gesellschaft zu Jena, 1886. Also a separate. 24 pp. 8vo.

('88). Soyka, J., und Bandler, A. Die Entwickelung von (pathogenen) Spaltpilzen unter dem wechselseitigen Einfluss ihrer Zersetzungsprodukte. Fortschr. d. Med. Bd. VI, 1888. pp. 769-773.

Treats of growth of bacteria in media exhausted for other organisms.

('88). Banti, Guido. Sulla distruzione dei batterii nell'organismo. Arch. per lo sc. med., 1888. Vol. XII, pp. 191-221, with 2 pp. of bibliography, 1 plate.

('89). Vignal, William. Contribution à l'étude des Bactériacées. Le mesentericus vulgatus. Paris, 1889.

('90). Koch, R. Ueber bakteriologische Forschung. Berlin, 1890, Verlag von August Hirschwald. pp. 15.

('93). Dixon, H. H. On the germination of seeds in the absence of bacteria. Sci. Trans. Roy. Dublin Soc., vol. v, series II, 1893-1896. pp 1-4. Review in Rev. Sci., 1894, pp. 437-438.

('94). Kochs, W. Giebt es ein Zellleben ohne Mikroorganismen? Biol. Centralb., 1894. No 14, pp. 481-491.

Answer, yes. Plants are grown from sterilized seeds and kept fourteen months without contact with bacteria. Plums refused to decay when the surface had been sterilized.

('94). Frankland. Die Bakteriologie in einigen ihrer Beziehungen zur chemischen Wissenschaft. Centralb. f. Bakt., Bd. XV, 1894, pp. 101-112.

('95). Ward, H. Marshall. On the biology of Bacillus ramosus, a schizomycete of the River Thames. Proc. Royal Soc. of London, vol. LVIII, 1895. 8vo. p. 265. Also a separate.

('95). Pfeffer, W. Ueber Election organischer Nährstoffe. Pringsheim's Jahrbücher, Bd. XXVIII, 1895, pp. 205-268.

('95). Achard, Ch., et Phulpin, E. Contribution a l'étude de l'envahissement des organes par les microbes pendant l'agonie et après la mort. Arch. de méd. exper. Tome VII, 1895, pp. 25-47.

('95). Beco, L. Etude sur la pénétration des microbes intestinaux dans la circulation générale pendant la vie. Ann. de l'Inst. Pasteur, T. IX, 1895, pp. 199-209.

('95-'96). Nuttall, George H. F., und Thierfelder, H. Thierisches Leben ohne Bakterien im Verdauungskanal. Ztschr. f. physiol. Chem. Bd. XXI, 1895, pp. 109-121, und Bd. XXII, Hft. 1, 1896, pp. 62-73.

('96). Lunt, Joseph. On Bacillus mesentericus niger (a new potato bacillus). Centralb. f. Bakt. 2. Abt., Bd. II, 1896. pp. 572-573.

Motile, liquefies gelatin rapidly, produces endospores copiously, blackens potato, curdles milk with subsequent solution of the curd, converts potato starch into sugar, i. e., there is no iodine reaction after a time, but a copious reduction of Fehling's solution.

('98). Sanarelli, G. Das myxomatogene Virus. Beitrag zum Studium der Krankheitserreger ausserhalb des Sichtbaren. Centralb. f. Bakt., XXIII Bd., 1898, pp. 865-873.

Author thinks it improbable that there are unorganized causes of infection, and that therefore certain infectious diseases must be due to organisms too small to be visible to the human eye, even when helped by the best optical appliances.

('99). Omélіansky, V. Sur la culture des microbes nitrificateurs du sol. Arch. d. Sci. biol. St. Pétersb. Tome VII, No 4, 1899, pp. 291-302.

('99). Sturgis, W. C. A soil bacillus of the type of de Bary's B. megaterium. Phil. Tr. Roy. Soc. of London. Series B, vol. 191, pp. 147-169. pl. 14-16, B. 172. London, 1899.

Organism described as Bacillus hortulanus.

('00). Hof, A. C. Untersuchungen über die Topik der Alkalivertheilung in pflanzlichen Geweben. Botanisches Centralb., Bd. LXXXIII, No. 9, XXI Jahrg., No. 35, 1900, pp. 273-280.

('00). Smith, R. Greig. The (bacterial) clouding of white wine. Proceedings of the Linnean Society of New South Wales, 1900. Part 4, Oct. 31. pp. 650-658. Also a separate.

('00). Jordan, Edwin O. Some observations upon the bacterial self-purification of streams. Jour. Exp. Med., vol. v, pp. 271 to 314. Dec., 1900. 1 plate.

('00). Ford, Wm. W. Varieties of colon bacilli isolated from man. Montreal Medical Journal, Nov., 1900 pp. 835-844 Also a separate, 10 pp. Bibliography of 14 titles.

('00). Smith, Theobold. Adaptation of pathogenic bacteria to different species of animals. Phila. Med. Journ., May 5, 1900. Vol. v., pp. 1,018-1,022.

('01). Fischer, Alfred. Ueber Protoplasmastruktur. Antwort an O. Buetschli. Archiv f. Entwickelungsm. d. Org., 1901, Bd. XIII, pp. 1-33.

('02). Joest, Ernst. Unbekannte Infektionsstoffe. Centralb. f. Bakt., Abt. 1, Bd. XXXI, Originale, 1902, pp. 361-384, pp. 410-422. Bibliog. of 58 citations.

('03). Ford, W. W. The classification and distribution of the intestinal bacteria in man. Studies from the Royal Victoria Hospital, Montreal, vol. 1, No. 5 (Pathology II). May, 1903, 95 pp.

('03). Winslow, C. E. A., and Nibecker, C. P. The significance of bacteriological methods in sanitary water analysis. Technology Quarterly of Mass. Institute of Technology, vol. XVI, No. 3, Sept., 1903, pp. 227-239. Also a separate.

('04). Phillips, Orville P. A comparative study of the cytology and movements of the Cyanophyceae (Plates XXIII-XXV, pp. 237-335. Bibliog. Trans. and Proc. Bot. Soc. Pennsylvania, Vol. 1, No. 3, 1904.

Finds short cilia on side walls of Cyanophyceæ.

VI. Important Books and Papers on Special Human and Animal Diseases.

('63). Davaine, C. Recherches sur les infusoires du sang dans la maladie connue sous le nom de sang de rate. C. R. des sé. de l'Acad. des sci., 1863, T. LVII, pp. 220-223, 351-353, 386-387.

This and many other papers on anthrax are reprinted in "l'Oeuvre de Davaine," Paris, 1889, 1 vol. 8vo.

('64). Davaine, C. Nouvelles recherches sur la maladie du sang de rate considérée au point de vue de sa nature. C. R. des sé. et mém. de la soc. d. biol. for 1863, Paris, 1864, pp. 193-202.

('70). Pasteur, Louis. Etudes sur la maladie des vers à soie, moyen pratique assuré de la combattre et d'en prévenir le retour. Tome I, La pébrine et la flacherie, pp. XII, 322, illustrated, and Tome II, Notes et documents, pp. 327, Paris, 1870. Gauthier-Villars.

('76). Koch, Robert. Aetiologie der Milzbrandkrankheit begründet auf die Entwicklungsgeschichte des Bacillus anthracis. Cohn's Beiträge z. Biol. d. Pflanzen. Bd. II, p. 277, Breslau, 1876.

('77). Pasteur, L. Étiologie des maladies charbonneuses. Archives Vétér. T. II, Paris, 1877, pp. 668-671.

('80). Pasteur, Chamberland et Roux. Sur l'étiologie du charbon. Archives Vétér. T. V., Paris, 1880, pp. 548-557.

('80). Pasteur, Louis. Sur les maladies virulentes, et en particulier sur la maladie appelee vulgairement choléra des poules. C. R. des sé. de l'Acad. des sci. T. XC, 1880, pp. 239-248.

('81). Koch, Robert. Zur Aetiologie des Milzbrandes. Mitteil. a. d. K. Gesundheitsamte. Bd. I, 1881, pp. 49-79.

('82). Loeffler und Schuetz. Vorläufige Mittheilung über die Arbeiten des K. Gesundheitsamtes, welche zur Entdeckung des Bacillus der Rotzkrankheit geführt haben. Deutsch. med. Wochenschr. Bd. VIII, 1882, pp. 707-708.

On the bacillus of glanders. See Vol CXV, New Sydenham Society for an English translation.

('83). Eberth, C. J. Der Typhusbacillus und die intestinale Infection. Vortrag 226, Volkmann's Sammel. Klin. Vorträge. Innere Med. No. 77, pp. 2,033-2,050, with 2 figs. Leipzig. Breitkopf und Härtel, 1883. Bibliography of 13 titles.

('83). Fehleisen. Die Aetiologie des Erysipels. Verlag von Theodor Fischer. Berlin, 1883, pp. 38.

('84). Pasteur, Chamberland et Roux. Sur la rage. C. R. des sé. de l'Acad. des Sci. T. XCVIII, Paris, 1884, pp. 1229-1231.

Earlier papers will be found in the same journal, T. XCVIII, p. 457; T. XCV, p. 1187, and T. XCII, p. 1259.

('84). Loeffler, Friedrich. Untersuchungen über die Bedeutung der Mikroorganismen für die Entstehung der Diphtherie beim Menschen, bei der Taube und beim Kalbe. Mitteil. a. d. K. Gesundheitsamte. Bd. II, 1884, pp. 421-499.

('84). Gaffky. Zur Aetiologie des Abdominaltyphus. Mittheil. a. d. K. Gesundheitsamte, Bd. II, 1884, pp. 372-420.

('84). Koch, Robert. Die Aetiologie der Tuberkulose. Mitteil. a. d. K. Gesundheitsamte, Bd. II, 1884, pp. 1-88, with 10 plates.

See Vol. CXV, New Sydenham Society for English translation. This paper should be read by every student who desires to know how a good piece of investigation is conducted.

('84). Nicolaier, Arthur. Ueber infectiösen Tetanus. Deutsche med. Wochenschr., 1884. Bd. X, pp. 842-844.

('84). Koch, Robert. Vortrag über die Cholera. Berliner klin. Wochenschr. Nos. 31 and 32, pp. 477-483 and 493-503. Deutsche med. Wochenschr. Nos. 32 and 32*a*, 1884, pp. 499-507 and 519-523. Reprinted also in Fortschr. der Medicin, Bd. II, Beilage, Heft 16, 1884, pp. 121-134 and Heft 17, pp. 141-168.

Describes the "comma bacillus," now generally recognized to be the cause of Asiatic cholera.

('84). Koch, R. Ueber die Cholerabakterien. Deut. Med. Wochenschr., Bd. X, 1884, pp. 725-728.

('84). Chauveau, A., et Arloing, S. Etude expérimentale sur la septicémie gangréneuse. Bull. de l'Acad. de méd., Paris, 6 mai, 1884, 2e série, T. XIII, pp. 604-615.

('85). Nicolaier, Arthur. Beiträge zur Aetiologie des Wundstarrkrampfes. Inaugural-Dissertation, Göttingen, 1885, W. Fr. Kaestner, pp. 31.

('87). Nocard et Roux. Sur la culture du bacille de la tuberculose. Annales de l'Inst. Pasteur, T. I., 1887, pp. 19-29.

('88-'90). Roux, E., et Yersin, A. Contribution à l'étude de la diphthérie. Ann l'Inst. Pasteur. 1er mémoire, Tome II, 1888, pp. 629-661. 2e mémoire, Tome III, 1889, pp. 273-288. 3e mémoire, Tome IV, 1890, pp. 385-426.

('89). Behring. Beiträge zur Aetiologie des Milzbrandes. Ztschr. f. Hyg., Bd. VII, 1889, pp. 171-185.

('89). Kitasato, S. Ueber den Tetanus bacillus. Ztschr. f. Hygiene, Bd. VII, 1889, pp. 225-234, 1 plate.

('89). Hog cholera. Its history, nature, and treatment, as determined by the inquiries and investigations of the Bureau of Animal Industry, U. S. Dept. Agric. Gov't. Pr. Office, Washington, D. C., 1889, pp. 197, pl. 16.

Bulletin prepared in great part by Dr. Theobald Smith.

('90) Smith, Theobald. On the influence of slight modifications of culture media on the growth of bacteria as illustrated by the glanders bacillus. Journal of Comparative Medicine and Veterinary Archives, vol. XI, pp. 158-161.

('91). Kitasato, S. Experimentelle Untersuchungen über das Tetanusgift. Zeitschr. f. Hyg., Bd. X, 1891, pp. 267-305.

('91). Smith, Theobald. Special report on the cause and prevention of swine plague. Bureau of Animal Industry, U. S. Dept. of Agriculture. Bull. 6, 1891, pp. 166, 12 plates.

('91). Smith, Theobald. Zur Kenntniss des hogcholerabacillus. Centralb. f. Bakt., Bd. IX, pp. 253-257, 307-311, and 339-343.

('92). Pfeiffer, R. Vorläufige Mittheilungen über die Erreger der Influenza. Deut. med. Wochenschr., Bd XVIII, 1892, p. 28.

('93). Behring. Die Geschichte der Diphtherie. Leipzig, 1893.

Not seen.

('93). Brieger, Ludwig, and Cohn, Georg. Untersuchungen über das Tetanusgift. Zeitschr. f. Hyg, Bd. xv, pp. 1-10, 1893.

As small a quantity of the tetanus poison as 0.000,23 gram would be a lethal dose for a man weighing 70 kilos. This is an inference based on experiments with mice.

('93). Moore, Veranus A. Observations on the morphology, biology, and pathogenic properties of twenty-eight streptococci found in the investigation of animal diseases. Bureau of Animal Industry, U. S. Dept. of Agriculture, Bull. No. 3, 1893, pp. 9-30.

('93). Moore, V. A. Pathogenic and toxicogenic bacteria in the upper air passages of domesticated animals. Bulletin No. 3, Bureau of Animal Industry, U. S. Dept. Agric., 1893, pp. 38-48.

('93). Pfeiffer, R. Die Aetiologie der Influenza. Zeitschr. f. Hyg., Bd. xiii, 1893, pp. 357-386, 8 plates.

('94). Novy, F. G. Ein neuer anaërober Bacillus des malignen Oedems. Zeitschr. f. Hyg, Bd. xvii, 1894, pp. 209-233, 2 heliotype plates from photomicrographs by Dr. Pfeiffer.

('94). Kitasato, S. The bacillus of bubonic plague. The Lancet, London, 1894 (ii), pp. 428-430.

('95). Sieber-Schoumow, Mme. N. O. Contribution à l'étude des poissons venimeux. Sur le Bacillus piscicidus agilis, microbe pathogène pour les poissons. Arch. d. sci. biol., Tome iii, 1895, St. Pétersb., pp. 226-256, 1 colored plate, 7 figs.

Author isolated from dying fish and from the waters of the reservoir containing the sick fish, its inflow and outflow pipes, an anaerobic, gas forming (CO_2), motile, short organism (*Bacillus piscicidus agilis*), pathogenic to cold-blooded animals, especially fish and frogs. Organism coagulates milk and grows readily at 37.5° C. It was killed by a heating (5-10 min.) in river water at 68-70° C., and agar or gelatin cultures yielded the cholera red reaction with hydrochloric acid. The organism is also toxic to white mice, guinea pigs, rabbits, and dogs, but not to pigeons.

('98). Shiga, Kiyoshi. Ueber den Erreger der Dysenterie in Japan. Centralb. f. Bakt., 1 Abt., Bd. xxiii, 1898, pp. 599-600.

('98). Smith, Theobald. A comparative study of bovine tubercle bacilli and of human bacilli from sputum. The Jour. of Exper. Med., vol. iii, 1898, pp. 451-511.

('98). Loeffler. Bericht der Commission zur Erforschung der Maul- und Klauenseuche bei dem Institut für Infektionskrankheiten in Berlin. Erstattet an den Cultusminister von dem Vorsitzenden der Commission, Berlin, Aug. 12, 1898. Deutsche mediz. Wochenschr., 1898, No. 35, pp. 562-564. Also a separate. Reprinted in Centralb. f Bakt., 1 Abt., Bd. xxiv, 1898, pp. 569-574.

Organism passes through a Chamberland filter, and is invisible.

('98). Petruschky, J. Ueber Massenausscheidung von Typhusbacillen durch den Urin von Typhus-Rekonvalescenten und die epidemiologische Bedeutung dieser Thatsache. Centr. f. Bakt. 1 Abt., Bd. xxiii, 1898, pp. 577-583.

('98). Nocard, Ed., et Leclainche, E. Les maladies microbiennes des animaux. 3d ed., 1903. Tome i, pp. ii, 668; Tome, ii, pp. 645. Paris, Masson et Cie.

('98). Nocard et Roux. Le microbe de la péripneumonie. Bulletin de la Soc. Central de Méd. Vétérinaire. Recueil de Méd. Vétérinaire Annexe, Paris. Nouvelle sér., T. 16. Mar. 24, 1898, pp. 213-233. See also the Veterinary Journal, London, vol. xlvii, pp. 147-152.

Authors describe as the cause of pleuro-pneumonia in cattle an organism of very small size, not visible clearly even after staining. The serum from diseased foci is extremely virulent, but it is impossible to cultivate anything from this serum by any of the ordinary methods. Many bacteriologists have tried and failed, including Nocard and Roux These authors finally succeeded in cultivating it in bouillon in collodion sacks. The bouillon was inoculated with a little of the virulent serum and the sacks were then placed in the peritoneum of rabbits, where they were allowed to remain some weeks subject to osmosis The organism clouds the bouillon slightly, and is visible under high magnifications, in bright light, as innumerable, minute, bright, mobile points. This bouillon is capable of reproducing the disease, but is free from bacteria cultivable on ordinary media. Check sacks incubated in the peritoneum gave no such result, neither did sacks inoculated with *heated virus*. Collodion sack cultures incubated in the peritoneal cavity of animals were first used (?) by Metchnikoff, Roux and Salimbeni in their study of the toxin and antitoxin of cholera.

The authors finally succeeded in cultivating this organism outside of the animal body, by using a special bouillon and a special agar (see Les maladies microbiennes des animaux, 1903, T. I, p. 150). "Virulent albuminous liquids, pulmonary serum not diluted, or the Martin serum bouillon filtered through Chamberland or Berkefeld bougies, gives a sterile filtrate. On the contrary, after dilution of the same liquids in a non-albuminous medium, the microbe passes through the Berkefeld and the Chamberland bougie. Under these conditions the filtration enables one to obtain without difficulty a characteristic pure culture, even from impure products."

('98). Nocard et Roux. Le microbe de la péripneumonie. Ann. de l'Inst. Pasteur, 1898, T. xii, pp. 240-262.

('99). Gelpke, Theodor. Bacterium septatum und dessen Beziehungen zur Gruppe der Diphtherienbacterien (B. diphtheriae [Klebs-Löffler], B. pseudodiphtheriticum [Löffler] und B. xerosis). Arb. a. d. Bact. Institut der techn. Hochschule zu Karlsruhe, ii Bd, 2 Hefte, 1899. pp. 71-148 5 plates (40 photomicrographs) and 4 charts. Bibliog. of 45 titles.

('00). Welch, William H. Morbid conditions caused by the Bacillus aerogenes capsulatus. Phila. Med. Journ., vol. vi, 1900, pp. 202-216.

('00). Flexner, Simon. On the etiology of tropical dysentery. Phila. Med. Journ., vol. vi, 1900, pp. 414-424.

The author calls special attention to Shiga's results. Flexner studied this disease in the Philippines. He says-"That the bacillus is identical with the organism obtained by Shiga in the epidemic of dysentery which prevailed in Japan, there can be no reasonable doubt. In morphological, cultural, and pathogenic characteristics the two organisms are indistinguishable."

('00). Gorham, F. P. The gas-bubble disease of fish and its cause. U. S. Fish Commission, Bull. for 1899, pp. 33-37, 1900, Washington.

('00). Smith, R. Greig. A new bacillus pathogenic to fish. Proceedings, Linnean Soc., New South Wales for 1900. Sydney, 1901, vol. xxv, pp. 122-130. Two heliotype plates.

This is named Bacillus piscidus bipolaris, in allusion to the bipolar germination of its spores The organism is motile, and liquefies gelatin.

('00). Smith, R. Greig. A fish disease from George's River. Proc. Linnean Soc., New South Wales for 1900. Sydney, 1901, vol. xxv, pp. 605-609.

('99-'00). Moschcowitz, Alexis V. Tetanus. A study of the nature, excitant, lesions, symptomatology, and treatment of the disease, with a critical summary of the results of serum therapy. Studies from the Dept. of Pathology of the College of Physicians and Surgeons, Columbia University, New York. Vol. vii, 88 pp. A bibliography of 337 titles. 1899-1900.

('01). Schultz, N. K. De la vitalité du microbe de la peste bubonique dans les cultures. Arch. des Sci. Biol. publiées par l'Inst. Impérial de Méd. Exper. à St. Pétersbourg. T. viii, No. 4, 1901, pp. 373-389. 1 plate.

Organism not sporiferous. Author made various tests from old cultures kept in sealed tubes of bouillon, and concludes that under favorable conditions the pest bacillus may preserve its vitality and virulence for four years In its most modified form, he says, it exists in the state of very small round grains.

('01). Tartakowsky, G., et Dchounkowsky. Du microbe de la péripneumonie des boeufs. Arch. des sci. biol. publiées par l'inst. impér. de méd. expér. à St. Pétersbourg. T. viii, 1901, pp. 441-460, 6 figs.

Confirms the work of Nocard and Roux.

('02). Marsh, M. C. Bacterium truttæ, a new species of bacterium pathogenic to trout. Science, n. s., vol. xvi, No. 409, pp. 706-707, Oct. 31, 1902. Also a separate.

This organism browns nutrient agar. Its thermal death point is said to be 42° C.

('02). Shiga, K. Weitere Studien über den Dysenteriebacillus. Zeitschr. f. Hyg. Bd. xli, 1902, pp. 355-368.

('02). Moore, V. A. The pathology and differential diagnosis of infectious diseases of animals. Ithaca, N. Y., 1902. pp. xiv, 380. 73 figs. 8 plates.

('02). Koch, Robert. An address on the transference of bovine tuberculosis to man. Brit. Med. Jour., London, 1902, vol. 2, pp. 1,885-1,889.

('03). Marsh, M. C. A more complete description of Bacterium truttæ. Bull. U. S. Fish Commission for 1902. Washington, Govt. Printing Office, 1903, pp. 411-415, with two plates. Also a separate.

Grows well in media which is neutral or + 5, but there is little or no growth when the acidity is + 15. Growth is also inhibited by an alkalinity of — 5. Gelatin and blood serum are liquefied. There is no growth on potato unless it is first neutralized; then there is a scanty white growth. Milk is not coagulated, but it becomes fairly transparent after two weeks. It does not ferment glucose, lactose or saccharose. It reduces nitrates to nitrites and to ammonia It is not clearly mobile It does not produce indol or phenol. The optimum temperature is at or near 20° C. It is actively pathogenic to trout, especially brook trout, in which the disease was first observed.

('03). Monfallet, D. Bibliographie abrégée des infections. Paris et Santiago (Chili), Ch. Goffi, éditeur, 1903, pp. 1-65.

About 1000 classified references to papers on human and animal diseases of a communicable nature.

('04). Remlinger, P. Les travaux récent sur la rage Bull de l'Inst. Pasteur, 1904, T. 11, pp. 753-764.

"We have shown that if one filters an emulsion of rabies virus through a Berkefeld V bougie and then centrifuges the filtrate, the upper layers are deprived of virulence, but the virulence is kept in the lower layers. Barratt has obtained the same results with an unfiltered emulsion of a rabid brain."

('04). Flexner, Simon, Holt, L. Emmett, and assistants. Bacteriological and Clinical Studies of the Diarrheal Diseases of Infancy, with Reference to the Bacillus Dysenteriæ (Shiga). Studies from the Rockefeller Institute for Medical Research, New York. Vol. ii, 1904, pp. 7-202.

VII. Predisposition, Conditions Favoring Infection or Immunity.

('80). Chauveau. See xxii.

('81). Loeffler, Friedrich. Zur Immunitätsfrage. Mitth. a. d. K. Gesundheitsamte. Bd. i, 1881, pp. 134-187. Also a separate, 54 pp.

('87). Metchnikoff. See xxi.

('88). Nuttall. See xxxviii.

('88). Nuttall. See xxi.

('88). Fluegge, C. Studien über die Abschwächung virulenter Bakterien und die erworbene Immunität. Zeitsch. f. Hygiene. 1888. Bd. iv, pp. 208-230.

('89). Buchner. See xxi.

('89). Nissen. See xxi.

('90). Lehmann. See xxi

('91). Ogata. See xxi.

('91). Buchner, Hans. Ueber Immunität, deren natürliches Vorkommen und künstliche Erzeugung. Münch. Med. Wochenschr. 1891, 38 Jahrg., pp. 551-554, 574-579.

('94). Ehrlich and Wassermann. See xxi.

('01). Hueppe, Ferdinand. Perlsucht und Tuberculose. Berliner klin. Wochenschrift, 1901, No. 34, pp. 876-878. Also a separate, pp. 10

('01). Zabolotny, D. Recherches sur la peste. 2 mém. Expériences d'inoculation, d'immunisation et de traitement des animaux. Arch. des Sci. Biol. publiées par l'Inst. Impérial de Med. Exper. à St. Pétersbourg. T. viii, No. 4, 1901, pp. 390-427. 2 plates.

('01). Metchnikoff, Elie. L'immunité dans les maladies infectieuses. Paris (Masson), 1901, pp. ix, 600, av. fig. German trans. by Julius Meyer. Jena (Fischer), 1902, pp. xi, 456.

Not seen.

('02). Ammon, Otto. Theoretische Betrachtungen über Ansteckung und Disposition. Arch. f. Hyg. Bd. xlii, 1902, pp. 289-305.

('02-'04). Kolle and Wasserman. See iii.

VIII. Symbiosis, Antagonism.

('81). Kern, Edward. Ueber ein neues Milchferment aus dem Kaukasus Bull. de la Soc. Imp. d. Nat. de Moscou, Tome lvi, pp. 141-177, 2d part (No. 3), 2 plates, 1881.

Kephir. Dispora caucasica, nov. gen. and n. sp. Plates good.

('84). Krannhals, H. Ueber das Kumys-ähnliche getränk "Kephir" und ueber den "Kephir"-pilz. Deutsch. Archiv. f. klin. med. Bd. xxxv, pp 18-37, 1 plate. Bibliography of 18 titles.

('84). Struve, Heinrich. Ueber Kephir Ber. d. deutsch. chem. Gesellsch. Bd. xvii, 1884, pp. 314-316 and 1,361-1,368.

('87). Metchnikoff. See xxi.

('87. Garré, C. Ueber Antagonisten unter den Bacterien. Correspondenzbl. f. Schweizer. Aerzte, Jahrg xvii, 1887, pp. 385-392

('88). Nuttall. See xxi.

('88). de Freudenreich, E. De l'antagonisme des bactéries et de l'immunité qu'il confère aux milieux de culture Ann. de l'Inst. Pasteur, T. ii, 1888, pp. 200-206.

('88). Dubois. See xxvii.

('88). Héricourt. Des associations microbiennes. Rev de méd., T. —, 1888, pp. —.

Not seen.

('88). Sirotinin. Ueber die entwicklungshemmenden Stoffwechselproducte der Bacterien und die sog Retentionshypothese. Zeitsch. f. Hyg. Bd iv, 1888, pp. 262-290.

('89) Beyerinck, M W. Sur le kéfir. Arch. néer des sci. ex. et nat, T. xxiii, 1889, pp. 428-444 1 fig.

('89). Rogers, G. H. Quelques effets des associations microbiennes. C. R. hebd d sé. et mém. de la soc. de. biol., 19 janvier, 1889. Paris, sé 9. T. 1, pp. 35-38.

Two bacteria, inoffensive to a given animal, may become pathogenic when inoculated together.

('90). Blagovestchensky, N. Sur l'antagonisme entre les bacilles du charbon et ceux du pus bleu. Ann. de l'Institut Pasteur, T. iv, 1890, pp. 689-715.

('91). Mix, Charles L. On a kephir-like yeast found in the United States. Proc. Amer. Acad. of Arts and Sciences, 1891, n. s. vol. xviii, pp. 102-114

('93). Ward, H. Marshall. The ginger-beer plant, and the organisms composing it. A contribution to the study of fermentation-yeasts and bacteria. Phil. Trans. Roy. Soc. (B) for 1892. London, 1893, vol. 183, pp. 125 to 197. Pl. 6.

('94). Galtier, V. Nouvelle recherches sur l'influences des associations bactériennes. Exaltation de la virulence de certain microbes. Accroissement de la receptivité. C R. des sé. de l'Acad. d. sci., T. cxviii, 1894, pp. 1,001-1,004.

('96?). von Freudenreich, Eduard. Bakteriologische Untersuchungen über den Kefir Landw. Jahrbuch d Schweiz, 1896. Bd. x, pp 1-20. 2 text figs. and 1 heliotype plate.

('97). von Freudenreich, Ed. Bakteriologische Untersuchungen uber den Kefir. Centralb. f. Bakt., 2 Abt. Bd. iii, 1897, pp 47-54. 2 figs; 87-95. 135-141

('00). Krause. See xv.

IX. Carriers of Infection.

('88). Alessi, Guiseppe. Sulla trasmissibilità dei germi infettivi mediante le deiezioni delle mosche. Arch. per lo sci. med. Vol. xii, Torino, 1888, pp. 279-292.

('91). Waite, M. B. Results from recent investigations in pear blight Proc. Am. Asso. Adv. Sci, 40th meeting. Salem, 1892.

Mr. Waite obtained his first results in 1891, and called attention to them at the Washington meeting of the Am. Asso. Adv. Sci. that summer.

('94). Yersen, La peste bubonique à Hong-Kong. Ann. de l'Inst. Pasteur. T. viii, 1894, pp. 662-667.

The pest is carried by rats.

('95). Smith, Erwin F. Bacillus tracheiphilus, etc. Centralb. f. Bakt.,etc. 2 Abt. 1 Bd., 1895, p. 365

The disease is spread by beetles.

('96). Smith, Erwin F. A bacterial disease of the tomato, egg plant, and Irish potato. Washington, 1896, p. 22, and Pl. ii, fig. 3.

Disease communicated by beetles.

('97) Marpmann. See xlix.

('97). Smith, Erwin F. Pseudomonas campestris (Pammel). The cause of a brown rot in cruciferous plants. Centralb f. Bakt. 2 Abt, Bd. iii, pp. 400-410.

Disease communicated by slugs and by larvæ of the cabbage butterfly.

('98). Simond, P L. La propagation de la peste. Ann de l'Inst. Pasteur, T. xii, 1898, pp. 625-687. 5 figs.

('98). Nuttall, George H. F. Zur Aufklärung der Rolle, welche stechende Insekten bei der Verbreitung von Infektionskrankheiten spielen. Centralb. f. Bakt., Bd. xxiii, 1 Abt, 1898, pp. 625-635

('99). Nuttall, G H F. On the rôle of insects, arachnids, and myriapods as carriers in the spread of bacterial and parasitic diseases of man and animals. Johns Hopkins Hosp. Repts, vol viii, No. 1-2, pp. 1-154. 3 plates. Bibliography of 366 titles.

Noticed in Nature, Dec. 14, 1899.

('00) Galli-Valerio, Bruno. Les puces des rats et des souris jouent-elles un rôle important dans la transmission de la peste bubonique à l'homme? Centralb. f. Bakt, 1 Abt., Bd. xxvii, 1900, pp. 1-4. 3 figs.

('02). Galli-Valerio, Bruno.. The part played by the fleas of rats and mice in the transmission of bubonic plague Jour Trop. Med., London, vol. v, 1902, pp. 33-36

('04). Brenner, W. Die Schwarzfaule des Kohls Centralb. f. Bakt. 2 Abt Bd. xii, p. 729

Disease said to be communicated by aphides.

X. General Morphology of the Bacteria. Cytology.

('74). Billroth, Theodor. Untersuchungen über die Vegetationsformen von Cocco-bacteria septica und den Antheil welchen sie an der Entstehung und Verbreitung der accidentellen Wundkrankheiten haben. Berlin, 1874. Verlag von Georg Reimer. Quarto, pp. xiv, 244. 5 plates.

('77) Billroth, Th., and Ehrlich, F. Untersuchungen über Coccobacteria septica. Archiv. f. klinische chir. Bd. xx, pp. 403-433. 1 pl, Berlin, 1877.

('78) Hallier, Ernst. Die Plastiden der niederen Pflanzen, ihre selbstständige Entwickelung, ihr Eindringen in die Gewebe, und ihre verherende Wirkung. Leipzig, 1878, pp 92. 4 plates.

('82). Zopf, W. Zur Morphologie der Spaltpflanzen Spaltpilze und Spaltalgen. Leipsic, 1882, pp. vi, 74. Verlag von Veit and Comp. 7 plates.

('86). Hueppe, Ferdinand. Die Formen der Bakterien und ihre Beziehungen zu den Gattungen und Arten. 1886. pp. viii, 152, 24 figs. Wiesbaden. C. W. Kreidel's Verlag.

('88). Pommer, Gustav. Ein Beitrag zur Kenntniss der fadenbildenden Bakterien. Mitth. bot. Inst. Graz, Jena, 1888, pp. 93-112. 1 plate.

Bacillus brassicæ, a sporiferous form, isolated from an imperfectly sterilized decoction of cabbage leaf.

('89). Metchnikoff, El. Contributions à l'étude du pléomorphisme des bactéries. Ann. de l'Inst. Pasteur, T. iii, 1889, pp. 61-68. 1 plate.

('89) Ernst, Paul. Ueber Kern-und Sporenbildung in Bakterien. Zeitschr. f. Hyg. Bd. v, 1889, pp. 428-486. 2 plates.

('89). Babes, Victor. Ueber isolirt färbbare Antheile von Bakterien. Zeitschr. f. Hyg. Bd. v, 1889, pp. 173-190. 1 plate.

('89). Winogradsky, S. Pléomorphisme des bactéries. Ann. de l'Inst. Pasteur, T. iii, 1889, pp. 249-264.

('89). Metchnikoff, El. Note sur le pléomorphisme des bactéries. Ann. de l'Inst. Pasteur, T. iii, 1889, pp. 265-267.

('90). Almquist, E Untersuchungen über einige Bakteriengattungen mit Mycelien. Zeitsch. f. Hyg. Bd. viii, 1890, Heft 2, pp. 189-197

('90). Bütschli, O. Ueber den Bau der Bakterien und Verwandter Organismen. Leipzig, C. F. Winter, 1890, pp 37. 1 pl.

Maintains the existence in the bacteria of a body corresponding to a cell nucleus.

('91). Famintzin, A. Eine neue Bakterienform, Newskia ramosa. Bulletin de l'Acad. de St. Pétersbourg. Nouvelle série (ii), 1891, T. xxxiv, p. 481.

Not seen.

('91). Dangeard. See xxiii.

('91). Protopopoff. Sur la question de la structure des bactéries. Ann. de l'Inst. Pasteur, 1891, Tome v, p. 332-336.

('91). Zettnow, E. Ueber den Bau der Bakterien. Centralb. f. Bakt. x Bd., 1891, pp. 689-694. 1 Tafel.

('92). Zukal, Hugo. Ueber den Zellinhalt der Schizophyten. Ber. d. deutsch. bot. Gesellsch. x, 1892, pp. 51-55.

('92). Sjoebring, Nils. Ueber Kerne und Theilungen bei den Bakterien. Centralb. f. Bakt., xi Bd., 1892, pp. 65-68, with 1 colored Tafel

"In the bacterial body there may be demonstrated consequently, as in other cells, two components—nucleus and cell-body, which, however, can not always be distinguished."

('92). Thaxter, Roland. On the Myxobacteriaceæ, a new order of Schizomycetes. Botanical Gazette, vol. xvii, No. 12, pp. 389-406. Pls. xxii-xxv, 1892.

('92). Sauvageau, C., et Radais. Sur les genres Cladothrix, Streptothrix, Actinomyces, et description de deux Streptothrix nouveau. Ann.de l'Inst. Pasteur. T. vi, 1892, pp. 242-273. 1 plate.

('92). Foerster, F. Ueber eine merkwürdige Er-scheinung bei Chromatium Okenii Ehrbg. sp Centralb f. Bakt , xi Bd , 1892, pp. 257-264, mit 1 taf. (colored).

Author's figures show curious bands or bridges connecting the bacterial cells sidewise as well as end to end.

('92). Trambusti, A., and Galeotti, G. Neuer Beitrag zum Studium der inneren Struktur der Bakterien. Centralb. f. Bakt., xi Bd., 1892, pp. 717-722, mit 1 Taf. (colored).

('92). Klein, E. Zur Geschichte des Pleomorphismus des Tuberculoscerregers. Centralb. f. Bakt., xii Bd., 1892, pp. 905-906.

('92). Buetschli, O. Untersuchungen über mikroskopische Schäume und das Protoplasma. 4to, mit 6 lithogr. Taf. u. 23 Fig. im Text, so wie einem Atlas von 19 Mikrophotographieen. Leipzig, 1892. Rev. in Centralb. f. Bakt., Bd. xiii, 1893, pp. 436-438.

Again maintains the existence in bacteria of a Centralkoerper corresponding to a nucleus, and points out that Alfred Fischer's criticism of his former statements has no substantial basis. See especially pp. 75 to 79.

('94). Beyerinck, M. W. Ueber die Natur der Faden der Papilionaceenknöllchen. Centralb. f. Bakt , Bd. xv, 1894, pp. 728-732.

('94). Ilkewicz, W. Ueber die Kerne der Milzbrandsporen. Centralb. f. Bakt., Bd. xv, 1894, pp. 261-267, mit 1 Figur.

('95). Ward, H. Marshall. The formation of bacterial colonies. Annals of Botany, vol. ix, 1895, pp. 653-657.

('95). Ward, H. Marshall. A false bacterium. Annals of Botany, vol. ix, 1895, pp. 657-658.

('95). Wager, Harold. Preliminary note upon the structure of bacterial cells. Annals of Botany, vol. ix, 1895, pp. 659-661.

('95). Coppen-Jones, A. Ueber die Morphologie und systematische Stellung des Tuberkelpilzes und über die Kolbenbildung bei Aktinomykose und Tuberkulose. Centralb. f. Bakt , xvii Bd., 1895, pp. 1-16 and 70-76, with 1 plate.

('95). Bruns, Hayo. Ein Beitrag zur Pleomorphie der Tuberkelbacillen. Centralb. f. Bakt., xvii Bd , 1895, pp 817-826, with 8 figs.

('95). Lubinski, Ws. Zur Kultivierungsmethode, Biologie und Morphologie der Tuberkelbacillen. Centralb. f. Bakt., xviii Bd., 1895, pp. 125-128.

('95). Babes, V. Beobachtungen über die metachromatischen Körperchen, Sporenbildung, Verzweigung. Kolben-und Kapselbildung pathogener Bakterien. Zeitschr. f. Hyg. Bd. xx, 1895, pp. 412-437, 2 plates.

Fig. 18 *c* of Table XI is particularly instructive. This shows a true branching in the anthrax organism, but it is confined to the capsule.

('96). Coppen-Jones, A. Ueber die Nomenclatur des sog. "Tuberkelbacillus." Centralb. f. Bakt., 1 Abt., Bd. xx, 1896, No. 10-11, pp. 393-395.

('96). Kanthack, A. A. Ueber verzweigte Diphtheriebacillen. Centralb. f. Bkt., xx Bd., 1896, pp. 296-297.

('96). Zettnow. Bilder von Spirillum undula majus bei freiwilligem Absterben. Centralb. f. Bakt., xix Bd , 1896, pp. 177-180, with 1 plate, 16 figs.

('97). Thaxter, Roland. Contributions from the Cryptogamic Laboratory of Harvard University. xxxix. Further Observations on the Myxobacteriaceæ. Botanical Gazette, vol. xxiii, No. 6, 1897, pp. 393-411, 2 plates

('97). Marpmann, G. Zur Morphologie und Biologie des Tuberkelbacillus. Centralb. f. Bakt., xxii Bd., 1897, pp. 582-586, with 1 plate.

('97). Johan-Olsen, Olav. Zur Pleomorphismusfrage. Centralb. f. Bakt., 2 Abt, Bd. III, 1897, pp. 273-284, 2 plates.

Finds branching forms

('97). Kitt. Die Streptothrixform des Rotlaufbacillus. Centralb. f. Bakt., XXII Bd, 1897, pp. 726-732, with 4 figs.

('98). Schultz. See XXXVIII.

('98). Stolz, Albert. Ueber besondere Wachstumsformen bei Pneumo- und Streptokokken. Centralb f. Bakt., XXIV Bd, 1898, pp. 337-343, with 6 figs.

Figures look like involution forms.

('98). Ziemann, Hans. Eine Methode der Doppelfärbung bei Flagellaten, Pilzen, Spirillen und Bakterien, sowie bei einigen Amöben. Centralb. f. Bakt., XXIV Bd, 1898, pp. 945-955, 1 plate.

The author's figures show the body of the spirillum blue enclosing 1-5 carmin colored granules.

('98). Ruzicka, Vlad. Zur Frage von der inneren Struktur der Mikroorganismen. Centralb. f. Bakt., XXIII Bd., 1898, pp. 305-307, with 1 plate.

Finds granules, which take stains, in bacterial body reserves conclusions as to their nature, but thinks they are not pleomorphic or degeneration phenomena.

('98). Grassberger, R. Zur Frage der Scheinfädenbildung in Influenzaculturen. Centralb. f. Bakt., XXIII Bd., 1898, pp. 353-364, with 1 plate and 4 text figs.

('98). Burchard, Georg. Beiträge zur Morphologie und Entwickelungs-Geschichte der Bacterien. Arb. a. d. Bact. Institut der tech Hochschule zu Karlsruhe, II Bd., 1 Heft, 1898, pp 1-64, 2 pl.

Twenty new species are described—Bacterium pituitans, B. petittomaticum, B flexile, B. turgescens, B. brachysporum, B. implectans, B petroselini, B. angulans, Bacillus goniosporus, B. pectocutis, B. paucicutis, B. cylindrosporus, B. leptodermus, B bipolaris, B. loxosus, B myxodens, B. armoraciae, B idosus, B. loxosporus, B. cursor.

('98). Wagner, A. Coli- und Typhusbakterien sind einkernige Zellen. Centralb. f. Bakt., XXIII Bd., 1898, pp. 433-438, and pp. 489-492, with 2 plates and 6 figs.

('98). Craig, Charles F. The branched form of the bacillus tuberculosis in sputum. The Journ of Exp. Med., vol. III, 1898, pp. 363-370, 1 plate.

Author thinks it is premature to separate B. tuberculosis from the bacteria on account of this phenomenon.

('99). Schulze, Otto. Untersuchungen über die Strahlenpilzformen des Tuberculoseerregers. Zeitschr. f. Hyg. Bd. XXXI, 1899, pp. 153-186, 1 plate.

('99). Galli-Valerio, Bruno. Contribution à l'étude de la morphologie du Bacillus mallei. Centralb. f. Bakt., XXVI Bd., 1899, pp. 177-180, with 5 figs.

Author finds branched forms in bouillon and on agar.

('99). Berestnew, N. Zur Frage der Klassifikation und systematischen Stellung der Strahlenpilze. Centralb. f. Bakt., XXVI, 1899, p. 300

('99). Moeller, Alfred. Ein neuer säure- und alkoholfester Bacillus aus der Tuberkelbacillengruppe, welcher echte Verzweigungsformen bildet. Centralb. f. Bakt., Bd. XXV, 1899, pp. 369-373, with 1 plate.

('99). Spirig, W. Die Streptothrix (Actinomyces) Natur des Diphtheriebacillus. Centralb. f. Bakt., XXVI Bd., 1899, pp. 540-541.

('99). Lubarsch, O. Zur Kenntniss der Strahlenpilze. Zeitschr. f. Hyg. Bd. XXXI, 1899, pp. 187-220, 1 plate.

('99). Marx, Hugo. Zur Morphologie des Rotzbacillus. Centralb. f. Bakt., XXV Bd., 1899, pp. 274-278, with 4 figs., showing branched forms.

('99) Muehlschlegel, A. Ein Beitrag zur Morphologie und Entwickelungsgeschichte der Bakterien nach Studien an drei Körnerbacillen. Arb. a d. kaiserl. Gesundheitsamte, Bd. XV, Heft. 1, pp. 131-152, 1899. 1 plate partly colored. Rev. in Centralb. f. Bakt., XXV Bd., 1899, p. 771.

73 titles cited under literature.

('00). Feinberg. Ueber den Bau der Bakterien. Centralb f. Bakt., XXVII Bd., 1900, pp. 417-426, with 5 plates.

Author believes he has demonstrated the existence of a nucleus in the bacteria by means of Romanowski's staining method (a mixture of methylene blue and eosin). The plasma stains blue; the nucleus, which may be small or which may fill nearly the whole bacterial body, stains red or red-brown.

('00). Zettnow. Romanowski's Färbung bei Bakterien. Centralb. f. Bakt., XXVII Bd., 1900, pp. 803-805.

Says Dr. Feinberg's papers contribute "nichts Neues"

('00). Nakanishi, K. Vorläufige Mitteilungen über eine neue Färbungsmethode zur Darstellung des feineren Baues der Bakterien. Münch. med. Wochenschr., 1900, No. 6. Rev. in Centralb. f. Bakt, XXVII Bd., 1900, pp. 547-549.

"All bacteria in their young stage, when they have grown under favorable conditions, are one-nucleate short cells."

('00). Skschivan, T. Zur Morphologie des Pestbakteriums. Centralb. f. Bakt., XXVIII Bd, 1900, pp. 289-292, with 4 text figs.

Finds V shaped and branched forms.

('00). Marx, Hugo, and Woithe, Friedrich. Morphologische Untersuchungen zur Biologie der Bakterien. Centralb. f. Bakt, XXVIII Bd, 1900, pp. 1-11, 33-39, 65-69, and 97-111, with 3 plates.

('00). Galli-Valerio, Bruno. Seconde contribution à l'étude de la morphologie du B. mallei. Centralb. f Bakt., XXVIII Bd., 1900, pp. 353-359, with 26 figs.

('01). Meyer, Arthur. Ueber die Verzweigung der Bakterien. Centralb. f. Bakt., 1 Abt., XXX Bd., 1901, No. 2, pp. 49-60, 2 plates.

('01). Rosenfeld. See XV.

('01). Reichenbach, H. Ueber Verzweigung bei Spirillen. Centralb. f. Bakt., 1 Abt, Bd XXIX, 1901, pp 553-557. 1 heliotype plate.

Many of the spirilla are shown with distinct branches.

('02). Buetschli, Otto. Bemerkungen über Cyanophyceen und Bacteriaceen. Archiv. f. Protistenkunde, Bd. 1, Heft. 1. Jena, Gustav Fischer, 1902. pp. 41-58, 1 plate.

On the nature of the "Centralkoerper."

('02). Errera, Léo. Sur une bactérie de grandes dimensions: Spirillum colossus. Recueil de l'Inst. botanique (Univ. de Bruxelles), T. V, 1902, pp. 347-357. Also a separate.

('02). LOEB, L. M. On branching forms of certain bacteria. Jour. of Med. Research. Vol. VIII, 1902 (n. s., vol. III), pp. 415-423.

The author finds branching forms in the typhoid bacillus.

('02). HEFFERAN, MARY. An unusual bacterial grouping. Centralb. f. Bakt., 2 Abt., VIII Bd., 1902, pp. 689-699. Also a separate, (with 5 figures in the text).

B. rosacens metalloides forms rosette-like groupings in liquid media and on some solid media.

('02). MATZUSCHITA, TEÏSI. Beobachtungen über den merkwürdigen Teilungsprocess bei einem proteusartigen Luftbacillus. (Vorl. Mitteilung). Centralb. f. Bakt., Abt. 2, Bd. IX, 1902, pp. 257-260, mit 1 Taf.

('03). HILL, H. W., AND RICKARDS, B. R. Notes on Morphology. I. "Snapping" as characteristic of the diphtheroid group. II. Branching of the organism of actinomycosis. Am. Pub. Health Ass'n. Proc. 30th Ann. meeting at New Orleans, Dec. 9-12, 1902, Columbus, Ohio, 1903. Also a separate, 4 pp., 2 figs.

('03). WOLBACH, S. B., AND ERNST, HAROLD C. Observations on the morphology of Bacillus tuberculosis from human and bovine sources. Jour. of Med. Research, 1903, vol. X, No. 3, pp. 313-333. 13 plates. Also a separate.

('04). THAXTER, ROLAND. Contributions from the cryptogamic laboratory of Harvard University, LVI, Notes on the Myxobacteriaceae. Bot. Gaz., June, 1904, vol. XXXVII, pp. 405-416, with 2 plates.

Cystobacter becomes Polyangium.

('04). LEPESCHKIN, W. W. Zur Kenntniss der Erblichkeit bei den einzelligen Organismen. Die Verzweigung und Mycelbildung bei einer Bakterie (Bacillus Berestnewi n. sp.). Centralblatt f. Bakt., 2 Abt., 1904, Bd. XII, pp. 641-648, and Bd. XIII, pp 13-22, 20 figs.

Describes a branched form.

XI. Spores.

[See also some papers under X, XV, and XXXIV.]

('76). COHN, FERDINAND. Untersuchungen über Bacterien. Beiträge zur Biologie der Pflanzen, Bd. II, Heft 2, 1876, pp. 249-276.

('84). BUCHNER, H. Ueber das Verhalten der Spaltpilz-Sporen zu den Anilinfarbstoffen. Aertzliche Intelligenzbl., Jahrg. XXXI, 1884, pp. 370.

('87). LEHMANN, K. B. Ueber die Sporenbildung bei Milzbrand. Munch. med. Wochenschr., 1887, pp. 485-488, No. 26.

('88). PRAZMOWSKI, A. Ueber Sporenbildung bei den Bakterien. Verhand. d. k. k. Akad. d. Wissenschaften in Krakau. Math.-naturw. Sektion, Bd. XVIII, 1888, p. 35, 1 Tafel.

Author thinks existence of "arthrospores" is not proved.

('88). GLOBIG. See XXXIII and XXXIV

('88). GRUBER. See XXXIII.

('88). BUCHNER, H. Ueber die vermeintlichen Sporen der Typhusbacillen. Centralb. f Bakt., 1888, Bd. IV, pp. 385-390

('89). KLEIN, LUDWIG. Ueber einen neuen Typus der Sporenbildung bei den endosporen Bacterien. Ber. d. deutsch. Botan. Gesellsch., Bd., VII, 1889, pp. (57)-(72). 1 plate.

Five spore-bearing bacilli are described. The spores are green. In one filamentous-septate species the spores generally occur in pairs, with a septum between, i. e., the spore is situated, if one may so speak, in the positive pole of one segment, and in the negative pole of its fellow.

('91). FISCHER. See XL.

('91). MOELLER, H. Ueber eine neue Methode der Sporenfärbung. Centralb. f. Bakt, Bd. X, 1891, pp. 273-277.

The author summarizes his method as follows. "The air-dried cover glass preparation is passed through the flame three times and put into absolute alcohol for two minutes, then two minutes in chloroform; then washed with water one-half to two minutes It is subsequently plunged into 5 per cent chromic acid and again thoroughly washed in water, after which carbol-fuchsin is dropped upon it and warmed over the flame to boiling for 60 seconds. The carbol-fuchsin is then poured off, the cover-glass plunged into 5 per cent sulphuric acid until it is bleached, and then thoroughly washed in water. The watery solution of methylen blue or malachite green is then allowed to act upon it for 30 seconds, after which it is washed off. The spores should then be visible as a dark red in a beautiful green or blue bacterial body."

('91). CRAMER, E. Die Ursache der Resistenz der Sporen gegen trockene Hitze. Arch. f. Hygiene, 1891, Bd. XIII, pp. 71-112.

Resistance is due to extreme dryness of the spores, and to the fact that all their water is hygroscopic water, which evaporates quickly in dry air, leaving presumably pure water-free albumen.

('92). FOTH. Zur Frage der Sporenfärbung. Centralb. f. Bakt., XI Bd., 1892, pp. 272-278.

Does not find Moeller's chromic acid method universally applicable. Author sometimes substitutes chloriodid zinc. He has also found hydrogen peroxide very useful. He also uses anilin water fuchsin. A pure, colorless, toluidin free anilin may be had from Joh. Wolff, in Breslau. In case of anthrax spores, the proper time of exposure is $1\frac{1}{2}$ minutes with chromic acid; 2 to $2\frac{1}{2}$ minutes with concentrated chlorzinc iodide solution, and 3 minutes with hydrogen peroxide. Here also the author says he obtained the best results with H_2O_2

('93). FIOCCA, R. Ueber eine neue Methode der Sporenfarbung. Centralb. f. Bakt., XIV, 1893, No. 1, pp. 8-9.

Cover-glass preparations are plunged 3 to 15 minutes (mostly 3 to 5 minutes) into steaming hot water containing 10 per cent ammonia water, to which has also been added 10 to 20 drops of some alcoholic anilin solution. Covers thus treated are then plunged for a moment into water containing 20 per cent sulphuric or nitric acid, and are subsequently exposed to a contrast stain. The stains recommended are gentian violet, fuchsin, methylene blue and safranin; the contrast stains are vesuvin, chrysoidin, methylene blue, malachit green or safranin. The preparations are said to be clear and satisfactory.

('93). PHYSALIX. See XXXIII.

('94). ERNST, PAUL. Farbungsversuche an Sporen mit Hilfe der Maceration. Centralb. f. Bakt., Bd. XVI, 1894, pp. 182- 184.

('95). MIQUEL, P., AND LATTRAYE, E. De la résistance des spores des bactéries aux temperatures humides égales et superieures à 100 degrees. Ann. de micrographie. Tome VII, 1895, p. 110, 158, 205. Rev. in Centralb. f. Bakt, XIX Bd., 1896, pp. 360-362.

('95). BUNGE, R. Ueber Sporenbildung bei Bakterien. Fortschr. d. Med., Bd. XIII, 1895, No 20 and 21. Rev. in Centralb. f. Bakt., XVIII Bd., 1895, p. 718.

('96). SCHREIBER, OSWALD. Ueber die physiologischen Bedingungen der endogenen Sporenbildung bei Bacillus anthracis, subtilis, und tumescens. Centralb. f. Bakt., XX Bd., 1896, 353-374 and 429-437.

Forty-five papers cited at close of this article.

('96). BUCHNER, H. Ueber die physiologischen Bedingungen der Sporenbildung beim Milzbrandbacillus. Centralb. f. Bakt., XX Bd., 1896, pp. 806-807.

('98). CATTERINA, G. Ricerche sull'intima struttura delle spore dei batteri. Separate from Atti d. Soc. veneto-trentina, vol. III, Fasc. 2, Padova, 1898, 10 pages, with 1 plate. Rev. in Centralb. f. Bakt., XXVI Bd., 1899, pp. 35-36.

Some evidence in favor of existence of a nucleus.

('98). MIGULA, W. Der Keimgehalt und die Widerstandsfähigkeit der Bakterien der animalen Lymphe. Arb. a. d. Bact. Institut der tech. Hochschule zu Karlsruhe, II Bd., 1 Heft, 1898, pp. 65-72.

('98). AUJESZKY, ALADAR. Eine einfache Sporenfärbungsmethode. Centralb. f. Bakt., XXIII Bd., 1898, pp. 329-331.

The unfixed covers are placed in a boiling hot ½ per cent solution of HCl for 3 to 4 minutes, then washed in water, dried, fixed, and stained with hot carbol fuchsin (three times over flame). Covers are then cooled, bleached in 4 to 5 per cent sulphuric acid, and counterstained 1 to 2 minutes in malachit green or methylene blue.

('99). STEPHANIDIS, PHILOPIMIN. Ueber den Einfluss des Nahrstoffgehaltes von Nahrböden auf die Raschheit der Sporenbildung und die Zahl und Resistenz der gebildeten Sporen. Arch. f. Hyg., Bd. XXXV, 1899, pp. 1-10. Review in Centralb. f. Bakt., XXVI Bd., 1899, p. 568.

In a poor substratum anthrax spores were formed more rapidly but in less numbers than in a rich medium. Toward heat the spores from the rich and poor media behaved alike.

('99). KLEIN, ALEX. Eine einfache Methode zur Sporenfärbung. Centralb. f. Bakt., XXV Bd., 1899, pp. 376-379.

Klein's modification consists in staining the spores before they have dried. In a watch glass he makes a spore emulsion in phys. salt solution. To this is added an equal volume of filtered carbol fuchsin. This is then gently heated over the open flame for six minutes, i. e., until steam rises. Covers are now prepared and the bacterial layer fixed by passing twice through the flame. The covers are then passed through 1 per cent H_2SO_4 for 1 to 2 seconds, washed in water and counterstained 3 to 4 minutes in alcoholic methylene blue solution diluted with water.

('99). DANNAPPEL. See XXXIII.

('00). SMITH, R. GREIG. The double staining of spores and bacilli. Proceedings of the Linnean Society of New South Wales, 1900, Part 3, June 27, pp. 394-397. Also a separate (issued Nov. 22, 1900).

('02). SCHAUDINN, FRITZ. Beiträge zur Kenntnis der Bakterien und verwandter Organismen. I. Bacillus butchlii, Arch. f. Protistenkunde, Bd. I, 1902, pp. 306-343, 1 plate. Bibliography of 24 titles.

This very large, slow-moving organism was isolated from the intestinal tract of a cockroach, Periplaneta orientalis.

The author states that this organism, like Kern's Dispora caucasica, is constantly disporous. B. buetchlii was selected for study of its inner structure, on account of its large size. The membrane did not give the cellulose reaction.

Seventy-three figures are given, illustrating inner structure, stages in the formation of the spores (one in each pole of the rod), polar germination of the spores, etc. The organism is 24 to 80 μ long by 3 to 6 μ broad, mostly 50 to 60 μ x 4 to 5 μ. The bacillus is flagellate, after the manner of B. subtilis.

XII. Flagella.

('38). EHRENBERG. See V.

('72). COHN. See V.

('75). DALLINGER, W. H., AND DRYSDALE, J. J. On the existence of flagella in Bacterium termo. The Monthly Microscopical Journal, Sept. 1, 1875, pp. 105-108.

('76). WARMING, EUG. Om nogle ved Danmarks Kyster levende Bakterier. Kjöbenhavn, 1876.

('77). KOCH. See LV.

('78). DALLINGER, W. H. On the measurement of the diameter of the flagella of Bacterium termo: a contribution to the question of the "Ultimate limit of vision" with our present lenses. Journ. Roy. Micros. Soc., vol. I, 1878, pp. 169-175, 2 plates.

From the mean value of 200 measurements (50 with each of 4 high-power objectives) Dallinger concludes that the diameter of the unstained flagellum of B. termo, in round numbers, is one-two hundred and four thousandth (1-204000) of an inch. This is equal to about one-eighth micron.

('79). VAN TIEGHEM, PHILIPPE EDOUARD LÉON. Sur les prétendus cils des bacteries. Bull. de la Société Botanique de France, 1879, T. XXVI, pp. 37-45.

Van Tieghem maintained that the flagella were moved from within the body of the bacterium, they themselves being inert gelatinous organs, and not vibratile cilia.

('89). LOEFFLER, F. Eine neue Methode zum Farben der Mikroorganismen, im besonderen ihrer Wimperhaare und Geisseln. Centralb. f. Bakt., VI Bd., 1889, No. 8-9, pp. 209-224, mit 8 Photogrammen.

('89). TRENKMANN. Die Färbung der Geisseln von Spirillen und Bacillen. Centralb. f. Bakt., VI Bd., Oct. 15, 1889, No. 16-17, pp. 433-436.

('90). MESSEA. See LVI.

('90). LOEFFLER, F. Weitere Untersuchungen über die Beizung und Färbung der Geisseln bei den Bakterien. Centralb. f. Bakt., Bd. VII, 1890, pp. 625-630.

('90). TRENKMANN. Die Färbung der Geisseln von Spirillen und Bacillen. II. Mitth. Centralb. f. Bakt., 1890, Bd. VIII, No. 13, pp. 385-389.

Covers on which the bacterial film is dried without heat are put for 6 to 12 hours in water containing 2 per cent tannin and 0.5 to 0.25 of one per cent hydrochloric acid. They are subsequently washed for one hour in iodine water, and then stained ½ hour in weak gentian violet anilin water, made as follows: Into a test tube holding 25 cc. put a few drops of concentrated alcoholic solution of gentian violet and add 10 cc. of distilled water. Then pour out about one-half of this and fill up with anilin water. The clear stain is said to color the flagella well on a feebly-stained background.

('91). HUMPHREY, J. E. Notes on Technique. II. Bot. Gazette, 1891, pp. 71-73.

Cilia of zoospores of algæ and fungi are stained very readily and sharply "in a drop of moderately strong solution (in 90 per cent alcohol) of Hanstein's rosanillin-violet, composed of equal parts of fuchsin and methyl violet," after first fixing them in a couple of drops of 1 per cent osmic acid solution.

('92). Strauss, I. Sur un procédé de coloration à l'état vivant des cils ou flagella de certaines bactéries mobiles. C. R. de la Soc. de biologie, 1892, No. 23, pp. 542-543. Also Bull. Méd., 1892, p. 1,003.

('93). Sclavo. Di un rapido processo per le colorazione delle ciglia di alcuni microorganismi. Ministera dell'intern. Laboratori scientifica della direzione di Santa-Roma.

Rev. in Centralb f. Bakt. Bd. xv. p. 507, 1893.

('93). Nicolle, M., et Morax, V. Technique de la coloration des cils, etc. Ann. de l'Inst. Pasteur, T. vii, 1893, No. 7, pp. 554-561.

('93). Moore, V. A. On the character of flagella on the Bacillus choleræsuis, B. coli communis, and the B.typhi abdominalis. Wilder Quarter Century Book, Ithaca, N. Y., 1893, pp. 339-363.

('93). van Ermengem, E. Nouvelle méthode de coloration des cils des bactéries. Trav. du Lab. d. Hygiène et de Bact. de l'Univ. de Gand, T. I., l. 3, 1893.

Original not seen. A very good method. Reviewed in Zeitschr. f. Wissensch Mikr. Bd. xi. 1894, pp. 98-99, and in Ann. de Micrographie, T. V., 1893, pp. 394-395.

('94). Hessert, W. Geisselfärbung ohne Beize. Centralb. f. Bakt., Bd. xvi, 1894, No. 8-9, pp. 346-347.

See also A simple stain for ciliated bacteria. Chicago Med. Recorder, 1894, pp. 240-242.

('94). Bunge, R. Ueber Geisselfärbung von Bakterien. Fortschr. d. Medizin, Bd. xii, 1894, No. 12, pp. 462-464.

('94). Bunge, R. Zur Kenntniss der Geisseltragenden Bakterien. Ibid., No. 17, pp. 653-670.

('94). Bunge, R. Weitere Mittheilungen über Geisselfarbung. Fortschr. d. Mediz., Bd. xii, 1894, pp. 929-935.

('95). Moore, Veranus A. On the nature of the flagella and their value in the systematic classification of the bacteria. Jour. Am. Pub. Health Asso., Oct., 1895, Ann. vol. xx, pp. 432-444, 3 plates.

('95). Ferrier. Considérations générales sur le pléomorphisme des cils vibratiles de quelques bactéries mobiles.—Archives de médicine expérimentale et d'anatomie pathologique. Paris, Série 1. T. vii, 1895, pp. 58-75. 1 plate.

('96). Loewit, N. Zur Morphologie der Bakterien. Centralb. f. Bakt., xix Bd., 1896, pp. 673-686, with 1 plate.

('96). Kanthack, A. A., and Connell, T. W. The flagella of the tetanus bacillus, and other contributions to the morphology of the tetanus bacillus. Jour. Path. and Bact, iv, 1896-97, pp. 452-459

('98). Bowhill, Th. Eine neue Methode der Bakterien-Geisselfarbung bei Gebrauch einer Orceïnbeize. Hyg. Rundschau, 1898, No. 1. Rev. in Centralb. f Bakt., xxiii Bd., 1898, pp. 667-668.

('98). Stephens, J. W. Van Ermengem's method of staining flagella; a modification The Lancet, 1898, Oct. 1. Rev. in Centralb f. Bakt., xxv Bd., 1899, p 302

Substitutes for nitrate of silver a [illegible] per cent largin solution. The flagellæ are said to be cleaner and more distinct.

('99). Welcke, E. Eine neue Methode der Geisselfärbung. Arch. f. klin. Chir., Bd. lix, 1899, Heft. 1, pp. 129-143. Rev. in Centralb. f. Bakt., Bd. xxvi, 1899, pp. 520-521.

A silver process of about the same complexity as that of van Ermengem.

('99). Zettnow. Ueber Geisselfarbung bei Bakterien. Zeitschr. f. Hyg., Bd. xxx. 1 Heft., March, 1899, pp. 95-106.

Discusses van Ermengem's silver method, and gives a gold method which is said to be better.

('99). Morton, N. Flagella staining. Trans. Jenner Inst., London, vol. 1. 2 series, pp. 242-243, 1899.

Tap water is recommended for dilutions. A 24-hour agar surface growth furnishes the bacteria. They are allowed to diffuse in a little of this sterilized water in a watch glass. A drop or two of this is then placed on the clean slide or cover and spread as widely as possible without use of the needle. The excess is absorbed by blotting paper. The dried film is not fixed by heat. The stain consists of tannic acid 1 gram, potash alum 1 gram, distilled water 40 cc. To this is added 0.5 gram of night blue dissolved in 20 cc of absolute alcohol. The copious precipitate which results is carefully removed by filtration. The fluid is then ready for use Stain 2 minutes. The flagella are blue, the body of the organism is not stained. Longer exposures cause precipitates Counterstain for the body by exposure for 1 or 2 minutes to anilin-water gentian violet. "I consider that the application of heat and the spreading of the film with the needle are very fatal to good results in flagella staining."

('00). Hinterberger, A. Eine Modifikation des Geisselfärbungsverfahrens nach van Ermengem. Centralb. f. Bakt., Bd. xxvii, No. 16-17, 1900, pp. 597-605, 1 plate and 1 fig.

('01). Williams, Hugh. Flagella stain. See Pathological technique, by Mallory and Wright. Second Ed., 1901. pp. 104-106. W. B. Saunders & Co., Philadelphia and London.

('02). Meyer, Arthur. Kurze Mitteilung über die Begeisselung der Bakterien. Centralb. f Bakt., Abt. 1, Bd. xxxi, Originale, 1902, pp. 737-739.

XIII. Capsules.

('78). Cienkowski. Untersuchung über die Gallertbildungen des Zuckerrübensaftes Resumé allemand du mémoire russe, Charkow, 1878.

Not seen.

('85). Ribbert. Zur Farbung der Pneumoniekokken. Deutsche med. Wochenschr., 11 Jahrg., 1885, p. 136.

Gives a method for staining capsules.

('85). Friedlaender, C. Ueber Pneumonie-Micrococcen. Fortsch. d. Med., Bd. iii, 1885, pp. 91-93.

Replies to criticism of Germain Sée, and gives his method of staining the capsule.

('85). Friedlaender, C. Notiz, die Farbung der Kapselmicrococcen betreffend Fortsch. d. Medicin, Bd. iii, 1885, No. 23, pp. 757-760.

The author's plan for staining capsules is as follows: Pass the dried covers three times through the flame, and then expose from one to several minutes in 1 per cent acetic acid water. Remove from the cover by blowing through a glass tube drawn to a point, and dry in air quickly. Then expose in a saturated solution of anilin-water gentian violet for a very few seconds, wash in water and examine. The exposure to the gentian violet should be barely long enough to stain the capsule without staining the interior protoplasm

('92). Liesenberg, C., u. Zopf, W. Ueber den sogenannten Froschlaichpilz (Leuconostoc) der europäischen Rübenzucker- und der javanischen Rohrzuckerfabriken. Zopf's Beitraege. Hft. i, 1892, pp. 1-29, with 2 plates, and Hft. ii, 1892, pp. 1-2. Rev. in Am Nat., March, 1897, p. 228.

('02). Welch, Wm. H. A gas-producing bacillus (B. aerogenes capsulatus, nov. spec.) capable of rapid development in the blood-vessels after death. Bull. Johns Hopkins Hospital, vol. 3, No. 24, July-August, 1892, pp. 81-91. Also a separate.

('94). Koch, Alfred, and Hosaeus, Hans. Ueber einen neuen Froschlaich der Zuckerfabriken Centralb. f. Bakt., Bd. xvi, 1894, pp. 225-228, mit 1 fig.

Describes a branching gelatinous organism as Bact. pediculatum. The branching is due to a terminal splitting or one-sided development of the capsule.

('95). Stift, A. Ueber die in den Produkten der Zuckerfabrikation auftretenden Bakterien. Centralb. f. Bakt., 2 Abt., Bd. i, 1895, pp 277-283.

('96). Wilde, Max. Ueber den Bacillus pneumoniæ Friedländer's und verwandte Bakterien. Inaugural Dissertation, Bonn, 1896, pp. 74. Bibliography of 89 titles. Printed by Carl Georgi.

('96). Fricke, Carl. Ueber den sogenannten Bacillus mucosus capsulatus. Zeitschr. f. Hyg., Bd. xxiii, 1896, pp. 380-451, with a bibliography of 25 titles.

('98). Pane, N. Ueber die Genesis der Kapseln des Pneumococcus. Centralb. f. Bakt., xxiv Bd., 1898, pp. 289-204, with 2 figs.

The capsule is the swollen outer part of the bacterium.

('98). Kaufmann. Eine neue Methode zur Färbung von Bakterienkapseln. Hyg. Rundschau, 1898, No. 18. Abstr. Centralb. f. Bakt., 1 Abt., Bd. xxv, p. 32

('99). Ward. See xlvii.

('99). Moore, A. Capsule staining. Trans. Jenner Inst., 2d ser., pp. 244, 1899.

Recommends a contrast stain for the capsule. The preparation is first fixed with dilute acetic acid, then stained in carbol fuchsin for about 1 minute, washed in distilled water and dried. It is then stained, with or without gentle heat, in night blue for 1 or 2 minutes, washed and dried. The night blue solution is Morton's modification of McCrorie's stain. (See Morton, Flagella staining.) By this method the capsule is stained blue and the body dark red.

('99). Strong, Lawrence Watson. Ueber die Kapselbacillen. Centralb. f. Bakt., xxv Bd., 1899, pp. 49-52.

('00). Boni, Icilio. Methode zur Darstellung einer "Kapsel" bei allen Bakterienarten. Centralb. f. Bakt., xxviii Bd., 1900, pp. 705-707.

('01). Ward. See xlvii.

('01) Boni, I. Ricerche sulla capsula dei batteri. Giorn Soc. ital. igiene, Milano, vol xxiii, 1901, pp. 417-430.

('02). Smith, R. Greig. An Ascobacterium from the sugar-cane, with notes upon the nature of the slime (Bacterium sacchari, n. sp.) Proceedings of Linnean Soc. of New South Wales, vol xxvii, 1902, part 1, pp. 137-145 1 plate. Also a separate (issued Aug 22, 1902). See also Centralb. f. Bakt., 2 Abt., Bd. ix, p. 806.

XIV. Stains and Staining Methods.

(See also XI, XII, and XIII.)

('--). Ehrlich. Beiträge zur Kenntniss der Anilinfärbungen und ihrer Verwendung in der mikroskopischen Technik. Arch. f. mikr. Anat. Bd. xiii, p. 263.

('75). Weigert. Färbung von Bakterien. Ber. über d. Sitzungen d. schlesischen Gesellsch. f. vaterl. Cultur, 10 Dec., 1875.

('77). Koch. See lv.

('81). Weigert. Zur technik der mikroskop. Bakterienuntersuchungen. Virch. Archiv. Bd. lxxxiv, 1881, p. 275.

('82). Ehrlich. Färbung der Tuberkelbacillen. Verhdlgn d. Ver. f. i. Med, 1 Mai, 1882 D. med. Woch., 1882, p. 269

('82). Ziehl. Zur Färbung des Tuberkelbacillus. Deutsche med. Wochenschr., 1882, No. 33, p. 451.

('82). Szyszylowicz, J. Das Corallin als mikrochemisches Reagens in d. Pflanzenhistologie, R. i. S. Ak. Krakau, T. x, 1883, pp. 97-114 Not seen. Rev Bot. Centralb, 1886, Bd xxviii, p. 51. See also Bot. Centralb., Bd. xii, 1882, p. 139.

This substance should be dissolved in sodium carbonate. It is used as a test for slime derived from starch, which stains diffusely purple or rose. After staining, the preparation should be subjected to hot alcohol, which removes the stain from all but the starch grains and slime derived therefrom. Gum slime and cellulose slime are bleached, the former even in cold alcohol. Pure gum does not stain. Mount the preparation in Canada balsam.

('83). Ziehl. Zur Lehre von den Tuberkelbacillen, insbesondere uber deren Bedeutung fur Diagnose und Prognose. Deutsche med. Wochenschr., 1883, p. 62.

('84). Koch. See vi.

('84). Loeffler. See vi.

('84). Gram, C. Ueber die isolirte Farbung der Schizomyceten in Schnitt-und Trockenpräparaten. Fortschr. d. Med., Bd. ii, No. 6, 1884, pp. 185-189.

('86). Pfeffer, W. Ueber Aufnahme von Anilinfarben in lebende Zellen. Untersuchungen a. d. bot. Inst. Tübingen, ii, p. 179, 1886.

('87). Unna. Die Rosaniline und Pararosaniline, eine bacteriologische Farbenstudie. Dermatologischen Studien. Heft 4, pp. 9-73. Hamburg, Voss, 1887.

('87). Kuehne. Ueber ein combinirtes Universalverfahren, Spaltpilze im thierischen Gewebe nachzuweisen. Dermatol. Studien, herausgeg. von Unna. Heft 6. Hamburg, L. Voss, 1887.

('87). Heim, L. Die Neuerungen auf dem Gebiete der bakteriologischen Untersuchungsmethoden seit dem Jahre, 1887, I. Farbungsmethoden. Centralb. f. Bakt., x Bd, 1891, pp. 260-265, 288-296, and 323-328.

('88) Kuehne, H. Praktische Anleitung zum mikroskopischen Nachweis der Bakterien im thierischen Gewebe. Leipzig (Günthers), 1888, pp 15-23. Methylenblaumethode.

('88). Unna, P. G. Die Entwicklung der Bakterienfarbung Eine historisch-kritische Uebersicht. Centralb. f. Bakt., 1888, iii Bd., pp. 22-26; also pp. 61-63, 93-99, 120-125, 153-158, 189-195, 218-221, 254-259, 285-291, 312-320, 345-348

At the end 71 papers on this subject are mentioned by title.

('91). Pregl, Fritz. Ueber eine neue Karbolmethylenblau-Method. Centralb. f. Bakt., x Bd., 1891, pp. 826-829.

Pregl finds the following method of staining sections on slides satisfactory: "Exposure for one-half to one minute to carbol methylen blue, sometimes with the aid of heat; second, brief washing in water; third, bleaching in 50 per cent alcohol until the section has become pale blue passing into greenish; fourth, removal of the water by means of absolute alcohol; fifth, clearing in xylol; sixth, enclosure in balsam."

Kuehne's carbol methylen blue is made as follows: 1.5 grms. methylen blue, 10 grms. absolute alcohol, 100 grams 5 per cent carbolic-acid water. The alcohol is poured over the methylen blue; the carbolated water is then added and rubbed up thoroughly with it. If only a small quantity is needed from time to time, it is better to make it up in less amounts, as, with time, the staining power of the solution decreases.

('92). Kuehne, H. Das Malachitgrün als Ausziehungsfarbe. Centralb. f. Bakt., xi Bd., 1892, pp. 756-758.

Author finds malachit green dissolved in anilin oil very useful for treatment of sections stained in carbol-fuchsin, etc. The bacteria stand out distinctly deep red on a bluish background. See paper for details.

('93). Nicolle, et Cantacuzène, J. Propriétés colorantes de l'oxychlorure de ruthénium ammoniacal. Ann. de l'Inst. Pasteur, T. vii, pp. 331-334.

('95). Unna, P. G. Ueber Verwendung von Anilinmischungen zur tinktoriellen Isolierung von Gewebselementen. Monatshefte f. prakt. Dermatologie, Bd. xxi, 1895. Rev. in Centralb. f. Bakt., i Abt., xx Bd., 1896, p. 406.

('95). Nicolle, M. Pratique des colorations microbiennes, Methode de Gram modifiée et méthode directe. Ann. de l'Inst. Pasteur, T. ix, 1895, No. 9. Rev. in Centralb. f. Bakt., xviii Bd., 1895, p. 552.

Carbol violet is substituted for anilin violet. Excess of color is removed with alcohol-aceton.

('97). Semenowicz, W., und Marzinowsky, E. Ueber ein besonderes Verfahren zur Färbung der Bakterien im Deckglaspraparate und in Schnitten. Centralb. f. Bakt., xxi Bd., 1897, pp. 874-876.

('00). Dreyer, Georges. Bakterienfärbung in gleichzeitig nach van Gieson's Methode behandelten Schnitten. Centralb. f. Bakt., i Abt., xxvii Bd., 1900, pp. 534-535.

Can be used for differential staining of animal tissues with bacteria in them, provided the latter are such as stain by Gram's method.

('00). Le Doux. Bemerkungen zu dem Artikel des Herrn M. Dorset: "A new stain for Bacillus tuberculosis." Centralb. f. Bakt., i Abt., xxvii Bd., 1900, p. 616.

Dorsett recommended Sudan III for staining the B tuberculosis. Le Doux says he has tried this repeatedly with entirely negative results.

('02). Levinson, Ja. B. Ueber Färbung des Fettes und der fettigen Degeneration der geformten Elemente in flüssigen und halbflüssigen Medien mit Sudan iii Wratsch. St. Petersberg, 1902, Bd. i, pp. 1,208-1,209 (Russian.)

('02). Grimme, Arnold. Die wichtigsten Methoden der Bakterienfärbung in ihrer Wirkung auf die Membran, den Protoplasten und die Einschlüsse der Bakterienzelle. Centralb. f. Bakt., Abt. i, Bd. xxxii, Originale, 1902, pp. 1-16, pp. 81-90, pp. 161-180, pp. 241-255, pp. 321-327, with 2 plates. Bibliog. of 78 titles.

XV. Morphological and Physiological Changes Due to Changed Environment.

('87). Schottelius. Untersuchungen über den Microc. prodigiosus. Festschr. f. Kölliker, Leipzig, 1887. Not seen.

This author obtained pigmented and non-pigmented races of Bacillus prodigiosus.

('89). Behring. Beitrage zur Aetiologie des Milzbrandes. vi. Ueber asporogenen Milzbrand. Zeitschr. f. Hyg., 1889, Bd. vii, pp. 171-176.

Author describes method of obtaining Aplanobacter (Bacillus) anthracis without spores.

('90). Smith. See vi.

('90). Roux, E. Bactéridie charbonneuse asporogène. Ann. de l'Inst. Pasteur, T. iv, 1890, pp. 25-34.

('92). Nencki, M. Ueber Mischkulturen. Centralb. f. Bakt., xi Bd., 1892, pp. 225-228.

('92). Adami, J. G. On the variability of bacteria and the development of races. Med. Chronicle, vol. xvi, 1892, No. 6, pp. 366-389. Also a reprint from Med. Chronicle, Sept., 1892, 8vo., 26 pp., Manchester (John Heywood), 1892.

('92). Charrin, A., et Phisalix. Abolition persistante de la fonction chromogène du Bacillus pyocyaneus.—C. R. des sé. de l'Acad. des Sci., Paris, 1892. T. cxiv, pp. 1,565-1,568.

('93). Sander. Ueber das Wachstum von Tuberkelbacillen auf pflanzlichen Nährböden. Arch. f. Hyg., Bd. xvi, Heft 3, 1893, pp. 238-311.

At 38°-39° C. (not at 22-23° C.), this author induced the organism of tuberculosis to grow on a variety of vegetable substances, i. e., potato, carrot, kohlrabi, macaroni, etc. All were cooked.

('93). Stoney, G. Suggestion as to a possible source of the energy required for the life of bacilli, and as to the cause of their small size. Proc. Roy. Soc., Dublin. Vol. viii, Pt. i, pp. 154-156. Dublin, 1893.

Views of a physicist.

('94). Surmont, H., et Arnould, E. Recherches sur la production du bacille du charbon asporogène. Ann. de l'Inst. Pasteur, T. viii, 1894, pp. 817-832.

('94). Ury, Jakob. Ueber die Schwankungen des Bacterium coli commune in morphologischer und kultureller Beziehung. (Inaug Diss.) 8vo, 47 pp., Strassburg i. E., 1894. Rev. in Centralb. f. Bakt., Bd. xvi, 1894, pp. 579-581.

('94). Smith, Theobald. Modification, temporary and permanent, of the physiological characters of bacteria in mixed cultures. Trans. Assoc. Amer. Physicians, Phila., 1894, pp. 85-109.

('94). Dieudonné, A. Beiträge zur Kenntnis der Anpassungsfähigkeit der Bakterien an ursprünglich ungunstige Temperaturverhältnisse. Arbeiten aus dem kaiserl. Gesundheitsamte, Bd. ix, 1894, pp. 492-508, Berlin, 1894 (Julius Springer). Also a separate Rev. in Centralb. f. Bakt., Bd. xvi, 1894, pp 965-967.

('95). Davenport, C. B., and Castle, W. E. On the acclimatization of organisms to high temperatures Archiv f. Entwickelungsmechanik der Organismen, Bd. ii, Heft 2, 1895, pp. 227-240. Bibliography of 3 pages.

Organisms become acclimatized to high temperature by losing water from their protoplasm.

('97). Peckham, Adelaide Ward. The influence of environment upon the biological processes of the various members of the colon group of bacilli. Jour. of Exper. Medicine, 1897, Sept., pp. 549-591.

('97). Auerbach, Wilhelm. Ursache der Hemmung der Gelatine-Verflüssigung durch Bacterien durch Zuckerzusatz. Arch. f. Hygiene, Bd. xxxi, 1897, pp. 311-318.

('98). Ficker, Martin. Ueber Lebensdauer und Absterben von pathogenen Keimen. (Habilitationsschrift.) Leipzig. Veit & Comp, 1898. Rev. in Centralb f. Bakt., xxvii Bd., 1900, p. 685.

Sorts of glass used for holding culture media have a distinct influence on certain bacteria by reason of substances dissolved out of it.

('98). Ruzicka, Syanislav. Experimentelle Studien über die Variabilität wichtiger Charaktere des B. pyocyaneus und des B. fluorescens liquefaciens. Centralb. f. Bakt., xxiv Bd, 1898, pp. 11-17.

('98). Niederkorn, Erminio. Vergleichende Untersuchung über die verschiedenen Varietäten des Bacillus pyocyaneus und des Bacillus fluorescens liquefaciens. (Inaug. Diss.). Freiburg, Switzerland, 1898. Rev. in Centralb. f. Bakt., xxvii Bd, 1900, pp. 749-750

('98). London, E. S. Le microbiomètre et son application à l'étude des phénomènes d'inanition chez les bactéries. Arch. des sci. biol. publiées par l'inst. imp. de méd. expér. à St. Pétersburg, T. vi, 1898, pp. 71-80.

The bacteria experimented on endured starvation only 49 to 88 days.

('99). Hellstroem, F. E. Zur Kenntnis der Einwirkung kleiner Glukosemengen auf die Vitalität der Bakterien. Centralb. f. Bakt., xxv Bd., 1899, pp. 170-180 and 217-223

Even small quantities of grape sugar proved harmful in case of acid-forming bacteria

('99). Madsen, Th. Einige Bemerkungen zu dem Aufsatz von F. E. Hellström zur Kenntnis der Einwirkung kleiner Glukosemengen auf die vitalität der Bakterien. Centralb. f. Bakt, xxv Bd., 1899, pp. 712-713.

('99). Tomasczewski, Egon. Ueber das Wachstum der Tuberkelbacillen auf kartoffelhaltigen Nahrboden. Zeitschr. f. Hyg. Bd. xxxii, 1899, Heft 2, p. 247.

('00). Kohlbrugge. See xlvi.

('00). Smith, Theobald. Variation among pathogenic bacteria. Jour. Bost. Soc. Med. Sci, vol. iv, No. 5, 1900, pp. 95-109.

('00). Emmerich, Rudolf, and Saida. Ueber die morphologischen Veränderungen der Milzbrandbacillen bei ihrer Auflösung durch Pyocyanase. Centralb. f. Bakt., xxvii Bd., 1900, pp. 776-787, with 1 colored plate.

('00). Krause, Paul. Beiträge zur Kenntnis des Bacillus pyocyaneus. Centralb. f. Bakt., xxvii Bd., 1900, pp. 769-775.

Considers effect of electricity (Tesla stream); pigment formation when in symbiosis with streptococci; behavior in hydrogen, carbon dioxide, illuminating gas, hydrogen sulphid; in vacuo; nature of the pigments.

('00). Smith, Theobald. Die Bedeutung von Varietäten bei pathogenen Bakterien. Centralb. f. Bakt., xxvii Bd, 1900, pp. 676-677.

('00). Matzuschita, Teïsi. Ueber die Veränderlichkeit der Eigenschaft des Bacillus anthracis, Gelatine zu verflüssigen. Centralb. f. Bakt., xxviii Bd., 1900, pp. 303-304.

('00) Matzuschita, Teïsi. Die Einwirkung des Kochsalzgehaltes des Nährbodens auf die Wuchsform der Mikroorganismen. Zeits. f. Hyg, 1900, Bd. xxxv, p. 495.

('01). Rosenfeld, A. Ueber die Involutionsformen einiger pestähnlicher Bakterien auf Kochsalzagar. Centralb. f. Bakt., 1 Abt., Bd. xxx, 1901, pp. 641-653.

('02). Smith, Theobald. The relation between bovine and human tuberculosis. The Medical News, New York, vol. lxxx, Feb. 22, 1902, pp. 342-346. Also a separate, pp. 14.

('02). Lepoutre, L. Recherches sur la transformation expérimentale de bactéries banales en races parasites des plantes. Annales de l'inst. Pasteur, T. xvi, 1902, pp. 304-312.

('04). Maassen, Albert Die teratologischen Wuchsformen (Involutionsformen) der Bakterien und ihre Bedeutung als diagnostisches Hilfsmittel. Arbeiten a. d. Kaiserlichen Gesundheitsamte, Berlin, 1904, Bd. xxi, Heft 3, pp. 385-400, pl. x to xv.

XVI. Culture-Media.

('59). Payen. Sur la gélose et les nids de salangane. C. R. des sé. de l'Acad. des sci, Paris, T. xlix, 17 Oct., 1859, pp. 521-530.

According to Payen, agar-agar has the following average composition: Carbon, 42.770; hydrogen, 5.775; oxygen, 51.445; total, 100.000.

('82). Naegeli, C. v. Untersuchungen ueber Niedere Pilze. a. d. Pflanzenphys. Inst. i. Muenchen. 1882, pp. 1-285.

('86). Guillebeau, A., et De Freudenreich, Ed. Préparation des gelées à base d'agar-agar. Archiv. des sci. phys. et nat., 3e Période, T. xv, Genève 1886, pp. 466-468.

This author describes a method of making agar without filtering. Cook for a quarter of an hour a water solution of agar (2 per cent), salt 0.5 per cent, and peptone 1 per cent. This is alkaline; neutralize. Add an equal quantity of bouillon prepared the day before. This bouillon contains salt and peptone in the same proportion (1 kilo. of meat for 2 litres of water). Cook in the autoclave for two hours at a temperatuoe of 120° to 125° C. Do not pass 130°, as agar changes color. Remove and let stand. After 4 to 5 hours one has in the upper part of the flask a limpid liquid with all impurities at the bottom. During this time the temperature must be kept above 42°. Decant and sterilize at 110° C.

('86). Miquel, P. "De la culture des bactéries" in Septième Mémoire sur les organismes microscopique de l'air et dex eaux. Annuaire de l'observ. à Montsouris pour 1885. See especially pp. 569-570.

This deals with use of Fucus crispus as basis for a solid culture-medium.

('87). Schottelius, M Einige Neuerungen an bacteriologischen Apparaten. 2 Vollständig klarer Agar-Nährboden. Centralb. f. Bakt., 1887, 11 Bd., pp. 100-101.

Agar is soaked about five minutes in 2 per cent hydrochloric acid and then washed in frequent changes of water. Five to ten per cent of this agar is then macerated over night in bouillon at room temperature; cooked; peptone and salt added; neutralized with carbonate of soda or potash; again cooked, and finally passed through filter paper.

('87). Dal Pozzo, D. Das Eiweiss der Kiebitzeier als Nährboden für Mikroorganismen. Med. Jahrb. Jahrg., 1887, pp. 523-529.

('87). Abbott, A. C. An improvement in the method of preparing blood serum for use in bacteriology. Medical News, 1887, vol. I, p. 207. Rev. in Centralb. f. Bakt., II Bd., 1887, pp. 424-425.

('87). Raskin, M. Zur Züchtung der pathogenen Mikroorganismen auf aus Milch bereiteten festen und durchsichtigen Nährböden, St. Petersburger med Wochenschr, XII Jahrg., 1887, pp. 357-360.

('88). von Freudenreich, E. Zur Bereitung des Agar-Agar. Centralb. f. Bakt., 1888, III Bd., pp. 797-798.

Recommends filtering nutrient agar in the autoclave at about 110°C. Time required 30 to 60 minutes.

('88). van Puteren. Ueber die Herstellung von festen Nährboden aus Milch zu Mikroorganismen Kulturen. (Russian.) 1888.

('88). Hueppe, Ferdinand. Ueber die Verwendung von Eiern zu Kulturzwecken. Centralb. f. Bakt., 1888, IV Bd., pp. 80-81.

Cultures are made in the uncooked eggs after shaking and proper surface sterilization.

('88). Roux, E. De la culture sur pomme de terre. Ann. de l'Inst. Pasteur, T. II, 1888, pp. 28-30, 2 figs.

('89). Petri, R. J. Ueber den Gehalt der Nährgelatine an Salpetersäure. Centralb. f. Bakt., V Bd., 1889, pp. 457-460.

Commercial gelatin generally contains nitrates in considerable quantities.

('89). Voigtlaender, Felix. Ueber die Diffusion in Agargallerte. Zeitschr. f. Physik. Chemie., Bd. III, 1889, pp. 316-335.

A study of some of the physical properties of agar. The rate of diffusion of many acids and other substances was determined. The conclusions are:

1. The diffusion in agar jelly from watery solutions is not disturbed by the process of imbibition.
2. The validity of Fick's law for dilute solutions was amply demonstrated.
3. The rapidity of diffusion of a substance in different concentrations of the agar jelly is the same. The diffusion constants observed in the jelly are like those of water or greater or less.
4. With rise of the temperature the constant does not increase in linear relation, but the amount of the salt which enters in increases.

('89). Rein, J. J. The industries of Japan; together with an account of its agriculture, forestry, arts, and commerce; from travels and researches undertaken at the cost of the Prussian Government; with 44 illustrations and 3 maps. London, Hudder & Stoughton, 27 Paternoster Row, 1889, pp. XII, 570. 2d revised German edition, 1905.

The cartilaginous Florideæ, particularly species and varieties of Gigartineæ, Caulacantheæ, Gelide, Sphærococceæ, and Tylocarpeæ, are distinguished for their high proportion of pararabin, and furnish, with boiling water algæ-jelly. They are gathered in great quantity on all the coasts of the Malay Archipelago and the waters of China and Japan, and are utilized in part direct as food, partly in the preparation of algæ-glue, Jap. Fu-nori, or algæ-jelly, Jap. Kanten. In trade these articles, both raw and dried, and when further prepared, are designated by the Malay word Agar-Agar, i. e., vegetable. This name was originally applied to Gigartina (Euchenma) isiformis, G. spinosa, and G. tenax, which is collected near Singapore, for example, in great masses, and shipped to China. The Chinese use them not only for food but make of them Hai-Thao, a transparent glue, with which they stiffen silk and other stuffs, and also fill up the interstices of coarse clothes for the manufacture of lanterns.

('89). Petri, R. J. Nachtrag zu "Ueber den Gehalt der Nährgelatine an Salpetersäure." Centralb. f. Bakt., V Bd., 1889, pp. 679-680.

('90). Kuehne, W. Kieselsäure als Nährboden für Organismen. Zeitschr. f. Biol. Neue Folge, Bd. IX, Der ganzen Reihe, Bd. XXVII, 1890, pp. 172-179.

('90). Tischutkin, N. Eine vereinfachte Methode der Bereitung von Fleisch-Pepton-Agar. Wratsch., 1890, No. 8, pp. 177-178. Reviewed in Centralb. f. Bakt., Bd. IX, p. 208, 1891.

The crude agar-agar is first exposed for 15 minutes in acetic-acid water (5 glacial acid, 100 water).

('91). Schultz, N. K. Zur Frage von der Bereitung einiger Nährsubstrate. Centralb. f. Bakt., X Bd., 1891, pp. 52-64.

('91). Sleskin, P. Die Kieselsaüre Gallerte als Nährsubstrat. Centralb. f. Bakt., Bd. X, 1891, pp. 209-213.

('91). Marpmann. Mittheilungen aus der Praxis. 1, Ersatz für Agar; 2, Ersatz für Gelatine. Centralb. f. Bakt., X Bd., 1891, pp. 122-124.

('92). Petri, R., und Maaszen, Albert. Ueber die Bereitung von Nährbouillon für bakteriologische Zwecke. Arbeiten aus d. kaiserl. Gesundheitsamte, Bd. VIII, No. 2, 1892, pp. 311-314.

('92). Loew, O. Ueber einen Bacillus, welcher Ameisensäure und Formaldehyd assimiliren kann. Centralb. f. Bakt., XII Bd., 1892, pp. 462-465.

('92). de Lagerheim, G. Macaroni als fester Nährboden. Centralb. f. Bakt., XI Bd., 1892, pp. 147-148.

Author states that cultures of chromogenic bacteria on macaroni stand out from the background very distinctly and are very instructive. The whitest macaroni should be selected. It is cut into pieces 4-5 cm. long. These are put into test tubes and covered 1 cm. over with water and cooked for 15 minutes. The water is then carefully poured off and the media sterilized in streaming steam in the usual way.

('92). Seiler, F. Influence de la composition de la gelatine nutritive sur le dévéloppement des colonies microbiennes. Schweizerische Wochenschr. f. Chemie u. Pharm, 1892, pp. 261-263.

('92). Schutz, J. Q. A rapid method of making nutrient agar. Bull. Johns Hopkins Hospital,vol. III, July-August, 1892, p. 92.

A useful and easy method.

('93). Uschinsky. Ueber eine eiweissfreie Nährlösung für pathogene Bakterien, nebst einigen Bemerkungen über Tetanusgift. Centralb. f. Bakt., Bd. XIV, 1893, No. 10, pp. 316-319.

('93). Nastiukoff. Ueber Nährböden aus Eigelb für Bakterienkulturen. Wratsch., 1893, No. 33 and 34. Rev. in Centralb. f. Bakt., XVII Bd., 1895, pp. 492-493.

('93). Hesse, W. Ueber den Einfluss der Alkalescenz des Nährbodens auf das Wachsthum der Bakterien. Zeitschr. f. Hygiene, Bd. XV, 1893, pp. 183-191. 3 plates

('94) Fraenkel, C. Beiträge zur Kenntniss des Bakterienwachsthums auf eiweissfreien Nährlösungen. Hyg. Rundschau, Jahrg. IV, 1894, pp. 769-776.

('94). WESNER, F. Die Bereitung eines festen undurchsichtigen Nahrbodens fur Bakterien aus Hühnereiern. Centralb. f. allg. Path. u. path. Anat., Bd. v, 1894, pp. 57-59.

Eggs are shaken until the yolks and whites are thoroughly mixed. They are then boiled hard. The shell is now removed, the egg cut into suitable pieces, put into test tubes and treated exactly like potato cylinders. This media is said to give very characteristic growths with many bacteria.

('95). TURRO, R. Ueber Streptokokkenzuchtung auf sauren Nährböden. Centralb. f. Bakt., Bd. XVII, 1 Abt., 1895, pp. 865-874.

('95). SEDGWICK AND PRESCOTT. See XLVI.

('95). ELSNER. Untersuchungen über electives Wachsthum der Bacterium coli-Arten und des Typhusbacillus und dessen diagnostiche Verwerthbarkeit. Zeitschr. f. Hyg., Bd. XXI, 1895, pp. 25-31.

('95). SMITH, THEOBALD. Ueber die Bedeutung des Zuckers in Kulturmedien für Bakterien. Centralb. f. Bakt., XVIII Bd., 1895, pp. 1-9.

('95). BLEISCH, MAX. See XVII.

('95). HAEGLER, CARL S. Zur Agarbereitung. Centralb. f. Bakt., XVII Bd., 1895, pp. 558-560, with 2 figs.

Advises centrifuging to clear the agar.

('95). DEYCKE, G. Die Benutzung von Alkalialbuminaten zur Herstellung von Nährböden. Centralb. f. Bakt., XVII Bd, 1895, pp. 241-245.

('95). FULLER, GEO. W. On the proper reaction of nutrient media for bacterial cultivation. Jour. Am. Pub. Health Asso., Oct, 1895, vol XX.

A very useful paper. It is recommended for general reading.

('95). HEIM, L. Zur Bereitungsweise von Nahrmitteln. Centralb. f. Bakt., XVII Bd, 1895, pp. 190-195, 1 phot.

('95). ZUPNIK, LEO. Zur Agarbereitung. Centralb. f Bakt., XVIII Bd., 1895, p. 202 Review in Bot Centralb., Bd. LXV, 1896, p. 52.

Author obtains clear agar by filtering it through a thin layer of absorbent cotton, placed funnel-form in the hot water filter, wet with hot distilled water and pressed in place with the fingers. The bouillon must be clear to start with. The agar powder is then added and cooked 1 hour in streaming steam

('95). MAASSEN, ALBERT. Die organischen Säuren als Nährstoffe und ihre Zersetzbarkeit durch die Bakterien. Arb. a. d. kaiserl. Gesundheitsamte, Bd. XII, Zweites Heft, 1895, pp. 340-411.

('96) CAPALDI, ACHILLE. Zur Verwendung des Eidotters als Nahrbodenzusatz. Centralb. f. Bakt, XX Bd., 1896, pp. 800-803.

('97). HESSE. See XLVI.

('97). MARPMANN. See XLIX.

('97). FORSTER, J. Nährgelatine mit hohem Schmelzpunkte. Centralb. f. Bakt, XXII Bd., 1897, pp 341-343.

By careful minimizing of heat the author obtains sterile nutrient gelatin melting at 29°-30° C

('97). STODDART, F. WALLIS. New method of separating the typhoid bacillus from the bacillus coli communis, with notes on some tests for the typhoid bacillus in pure cultures. University College, Bristol. The Jour. of Pathology and Bacteriology, IV, 1896-97, p. 429.

('97). BOKORNY, TH. Grenze der wirksamen Verdünnung von Nährstoffen bei Algen und Pilzen. Bio Centralb. June 15, 1897, pp. 417-420.

Author states that 0.002 per cent peptone serves no longer as a nutrient for bacteria, but 0.010 per cent does. In mineral solutions, monopotassium phosphate, magnesium sulphate, and calcium nitrate, a dilution to 0.001 per cent still nourishes algæ, but not bacteria, with 0.005 per cent solutions, bacteria appeared

('97). LONDON, E. S. Schnelle und leichte Methode zur Bereitung des Nahragars. Centralb f. Bakt, XXI Bd., 1897, pp. 686-687.

('97). HISS, PHILIP HANSON. On a method of isolating and identifying bacillus typhosus, based on a study of bacillus typhosus and members of the colon group in semi-solid culture media. Jour. Exp. Med., vol. II, 1897, pp. 677-700.

('98). SMITH, ERWIN F. Potato as a culture-medium with some notes on a synthesized substitute. Proc. Am. Asso. Adv. Sci, Vol. XLVII, 1898, p. 411. Also a separate. Centralb. f. Bakt, 2 Abt., Bd. V, p. 102.

('98) BOKORNY, TH. Sources of Carbon for Bacteria. See table by Bokorny in his Lehrbuch der Pflanzenphysiologie, pp. 56-59. Paul Parey, Berlin, 1898.

('98). GIESENHAGEN, K. Eine Vorrichtung zum Filtrieren von Nähragar. Centralb. f. Bakt., XXIV Bd., 1898, pp. 501-502.

('99). YOKOTE, T. Ueber die Darstellung von Nähragar. Centralb. f. Bakt., XXV Bd., 1899, pp. 379-380.

Author heats his filtered bouillon, to which agar has been added, for 1 hour on a sand bath, after which it filters readily if the sand temperature has been above 110° C. It will not filter satisfactorily if lower temperatures are used.

('99). BLIESENER. Ueber Gelatinekulturen im Brutschrank. Zeitschr. f. Hyg, Bd. XXXII, Heft 1, 1899. Rev. in Centralb. f. Bakt., XXVII Bd, 1900, pp. 472-473.

Author prepares a gelatin which remains solid at 27-30° C.

('99). CESARIS-DEMEL. Ueber das verschiedene Verhalten einige Mikroorganismen in einem gefarbten Nahr-Mittel. Centralb. f. Bakt., XXVI Bd, 1899, pp. 529-540, with 2 plates.

The medium recommended is liver broth with tincture of litmus This serves, it is said, to differentiate certain species.

('00). GLAESSNER, PAUL. Ueber die Verwertbarkeit einiger neuer Eiweisspräparate zu Kulturzwecken. Centralb. f. Bakt., XXVII Bd, 1900, pp. 724-732.

Comparative tests of somatose, nutrose, etc.

('02). YENDO, K. Uses of marine Algæ in Japan Postelsia, St. Paul, Minn., 1902, pp. 3-18. 3 plates and 3 Japanese prints

Gelidium corneum (Japanese Ten-gusa) furnishes the agar-agar of commerce.

('02). WHIPPLE, GEORGE C. On the physical properties of gelatin, with reference to its use in culture media. Technology Quarterly, Boston, Mass., vol. XV, pp. 127-160. Also a separate.

('04). GAGE, STEPHEN DE M., AND ADAMS, GEORGE O. Studies of media for the quantitative estimation of bacteria in water and sewage. Jour. of Infectious Diseases, vol. I, No. 2, 1904, pp. 358-377. Also a separate.

('04). HESSE, GUSTAV. Beiträge zur Herstellung von Nährböden und zur Bakterienzüchtung. Ztschr. f. Hyg., 1904, Bd. XLVI, pp. 1-22.

Discusses effect of adding various acids and alkalies, effect of heat on reaction, use of insoluble glass, etc. Most of the methods advised are already in use in many laboratories in the United States.

XVII. Methods or Work, Apparatus, Etc.

('73). KLEBS, E. Beitraege zur Kenntniss der Micrococcen. Arch. f. exp. Path. u. Pharmakol., 1873, Bd. 1, pp. 31-64, with 4 plates.

Klebs here outlines his fractional method of culture. See especially pp. 46-47.

('77). KOCH. See LV.

('78). LISTER, JOSEPH. On the lactic fermentation and its bearings on pathology. Trans. Path. Soc. of London, 1878, vol. XXIX, pp. 425-467.

Lister's dilution method for obtaining pure cultures is here described. See especially page 445 et seq.

('81). KOCH, ROBERT. Zur Untersuchung von pathogenen Organismen. Mittheil. a. d. kaiserlichen Gesundheitsamte, Bd. 1, 1881. Berlin, pp. 1-48, 14 plates.

In this paper Koch outlined his now universally used method of obtaining pure cultures (colonies) on solid media, viz: by use of gelatine for streaks and poured plates. See pp 24 and following, especially page 27. In 1881, or earlier, Buchner diluted his anthrax fluid until it was assumed that the quantity used to inoculate each flask contained only one organism (p. 19).

('83). KOCH, ROBERT. Ueber die Milzbrandimpfung, eine Entgegnung auf den von Pasteur in Genf gehaltenen Vortrag. Kassel und Berlin, Theodor Fischer, 1882. The same in French. Theo. Fischer, 1883.

The celebrated Koch "rules of procedure" are given in this paper.

('84). HOFFMANN, F. W. Einfacher Einbettungsapparat. Zoologischer Anzeiger, VII Jahrg., 1884, No. 157-184, Leipzig, pp. 230-232, 1 fig.

Makes use of a vacuum to hasten the infiltration of paraffin.

('84). ERRERA. Sur l'emploi de l'encre de Chine en Microscopie. Bull. Soc. Belge d. Microscop., 1884.

Not seen The ink is used to form a contrasting background.

('86). VON ESMARCH, ERWIN. Ueber eine Modifikation des Koch'schen Plattenverfahrens zur Isolierung und zum quantitativen Nachweis von Mikroorganismen.—Zeitsch. f. Hygiene, 1886, Bd. 1, pp. 293-301, 3 figs.

The method of roll cultures is here described.

('87). PETRI, R. J Eine kleine Modification des Koch'schen Plattenverfahrens. Centralb. f. Bakt., 1 Bd., 1887, No. 9, pp. 279-280.

Description of the now everywhere common Petri-dishes

('87). ESMARCH. See XXXIII

('89). BEYERINCK, M. W. L'auxanographie ou la méthode de l'hydrodiffusion dans la gélatine appliquée aux recherches microbiologiques. Arch. néerlandaises de sci ex. et nat., 1889, T. XXIII, pp 367-372

('90). KUEBLER. Untersuchungen uber die Brauchbarkeit der "Filtres sans pression, Systeme Chamberland-Pasteur." Zeitschr f. Hyg., Bd VIII, 1890, pp 48-54.

('91). NUTTALL, GEORGE H. F. A method for the estimation of the actual number of Tubercle bacilli in tuberculous sputum. With a note on the general application of the method to bacteriology. Bull. of the Johns Hopkins Hospital, vol. II, 1891, pp. 67-76.

By use of this method flasks or tubes of media can be inoculated, it is said, with predetermined exact numbers of bacteria.

('91). BUJWID, O. Eine einfache Filtervorrichtung zum Filtriren sterilisirter Flüssigkeit. Centralb. f. Bakt., IX Bd., 1891, pp. 4-5, mit 1 Abbildung.

('91). GABRITSCHEWSKY, G. Zur Technik der bakteriologischen Untersuchungen, I. Graduirte Kapillarpipetten zum Abmessen sehr kleiner Flüssigkeitsmengen. Centralb. f. Bakt., X Bd., 1891, pp. 248-250, mit 2 figs.

('91). NORDTMEYER, H. Ueber Wasserfiltration durch Filter aus gebrannter Infusorienerde.—Zeitsch. f. Hygiene, 1891, Bd. X, pp. 145-154, 1 fig.

('91). SMITH, THEOBALD. Kleine bakteriologische Mittheilungen. Centralb. f. Bakt., X Bd., 1891, pp. 177-186, mit 2 figs.

First two notes relate to apparatus; the second, to his inside-out method of filtering through Chamberland bougies (figured).

('91). KNAUER, FRIEDRICH. Eine bewährte Methode zur Reinigung gebrauchter Objektträger und Deckgläschen. Centralb. f. Bakt., Bd. X, 1891, pp. 8-9.

Even old stuck-together slides are said to be cleaned perfectly by boiling 30 minutes in 10 per cent lysol, with occasional stirring. They should then be flooded with tap water, and finally wiped with a soft, clean, oil-free cloth. "In recent preparations, 14 days old, I obtained even by 15 minutes boiling in 5 per cent lysol solution a perfect cleaning of the glass."

('91). ALTMANN, P. Thermoregulator neuer Konstruktion. Centralb. f. Bakt., IX Bd., 1891, pp. 791-792.

('91). BEYERINCK, M. W. Die Kapillarhebermikroskopirtropfenflasche. Centralb. f. Bakt., IX Bd., 1891, pp. 589-590, mit 1 Abbildung.

An easily constructed useful water flask, furnishing drops of any size (see fig 16) or a fine stream.

('92). HASWELL, WM. A. On a simple method of substituting strong alcohol for a watery solution in the preparation of specimens Proc. Linn. Soc. N. S. W., vol. VI, for 1891, pp. 433-436, 1 fig. Sydney, 1892.

This method was worked out 10 years later, independently, in my laboratory, by Deane B. Swingle, who had his paper and drawings ready for publication when this earlier paper was discovered. Swingle's device, which is a considerable improvement on Haswell's, is shown in Fig. 146. By means of the expanded base of the inner tube a quite equable diffusion of the alcohol is secured.

('92). GILTAY, E., u. ABERSON, J. H. Methode zur Prüfung von Filtereinrichtungen wie die Chamberland-Bougies. Centralb. f. Bakt., 1892, Bd. XII, pp. 92-95, 1 fig.

('92). V. FREUDENREICH, ED. Ueber die Durchlässigkeit der Chamberland'schen Filter für Bakterien. Centralb. f. Bakt., XII Bd., 1892, pp. 240-247.

The author's experiments lead to the conclusion that the Chamberland filter cannot be used safely more than a week for the continuous filtration of water It must then be sterilized.

('92) Smith, Theobald, and Moore, V. A. Zur Prufung der Pasteur-Chamberland Filter. Centralb. f. Bakt., XII Bd., 1892, pp. 628-629, mit 1 Abbildung.

('92). Altmann, P. Ein neuer Thermoregulator für Petroleumheizung bei Thermostaten. Centralb. f. Bakt., XII Bd., 1892, pp. 654-655, mit 2 figs.

('92). Arloing, G. De l'influence des filtres minéraux sur les liquides contenant des substances d'origine microbienne. C. R. des sé. de l'Acad. des sci., T. CXIV, 1892, pp. 1.455-1.457.

('92) De Freudenreich. De la perméabilité des filtres Chamberland à l'égard des bactéries. Annales de Micrographie, 1892, Tome IV, pp. 559-568.

('92). Holm, Just Chr. Sur les méthodes de culture pure et spécialment sur la culture sur plaques de M. Koch et la limite des erreurs de cette méthode. Meddelelser fra Carlsberg Lab. Tredie Bind. Andet Hefte. Kjobenhavn. 1892, pp 1-23. Danish edition, Tredie Bind. Forste Hefte, 1891, pp. 1-32

('92) Nencki and Zawadzki. See XLVII.

('92) Altmann, P. Neue Mikrogaslampen als Sicherheitsbrenner. Centralb. f. Bakt., XII Bd., 1892, pp. 786-787, 3 figs.

('93). Zettnow. Reinigung von neuen Deckgläsern. Centralb. f. Bakt., Bd. XIV, 1893, pp. 63-64.

The last traces of fat are best removed from covers by burning. The clean covers are placed on an 8 to 10 cm. square piece of sheet iron, which is then heated for a few minutes in the open flame of a Bunsen burner.

('93). Schepilewsky, E. A. Ein Regulator zum Thermostaten mit Wasserheizung. Centralb. f. Bakt., Bd. XIV, 1893, pp. 131-138, mit 1 fig.

('93). Gruber, Max. Gesichtspunkte für die Prüfung und Beurteilung von Wasserfiltern. Centralb f. Bakt., Bd. XIV, 1893. pp. 488-493.

('93). Kirchner, Martin. Gesichtspunkte für die Prüfung und Beurteilung von Wasserfiltern. (Entgegnung auf die gleichnamige Arbeit von Prof. Max Gruber in Wien). Centralb. f. Bakt., Bd. XIV, 1893, pp. 516-527.

('93). Brunner, G., and Zawadzki, A. Zählplatte zu den Petri'schen Schalen. Centralb. f Bakt., Bd. XIV, 1893, pp. 616-618, mit 1 fig.

A method is given for dividing a circle 10 cm. in diameter into 64 equal parts. The circles and sections are to be in white ink on black paper.

('93). Schoefer, Hans. Ueber das Verhalten von pathogenen Keimen in Kleinfiltern. Centralb. f. Bakt., Bd. XIV, 1893, pp. 685-693.

('93). Lafar, Franz. Eine neue Zahlvorrichtung fur Plattenkulturen in Petrischalen. Zeitsch. f. Nahrungsmitteluntersuchung u. s. w., Wien, 1893, No 24, p. 429. Rev. in Centralb. f. Bakt., Bd. XV, 1894, pp. 331-333, mit 1 fig

('94). Loeffler, F. Eine sterilisirbare Injectionsspritze. Centralb. f. Bakt., Bd. XVI, 1894, pp. 729-731.

To be had from Wittig, instrument maker in Greifswald, Germany.

('94). Houston, A. C. Note on a simple apparatus for collecting samples of water for bacteriological purposes, at different depths from the surface. Jour. of Path. and Bact., vol. II, 1894, pp. 496-497, 1 fig.

('94). Vallin, E. Le régénération par agents chimiques des filtres Chamberland. Revue d'hygiène et de police sanitaire, 1894, No. 11, p. 946. Rev. in Centralb. f. Bakt, XVII Bd., 1895, pp. 496-497.

Author recommends a sodium bisulphite solution (1:20) for cleaning filters.

('94). Kuprianow, J. Zur Methodik der keimfreien Gewinnung des Blutserums. Centralb. f. Bakt, XV Bd, 1894, pp. 458-462, 1 fig.

Describes and figures a device for filling test tubes with exact amounts of fluid culture media.

('94). Funck, Ernst. Zur Frage der Reinigung der Deckglaser. Centralb. f. Bakt., Bd. XVI, 1894, pp. 113-114.

Author cleans slides and covers which have been used with oil, Canada balsam, stains, etc., by first placing them for a time in turpentine, separating the covers from the slides as far as possible They are then put into a broad beaker, covered with hydrochloric acid, to which is added a few grams (2 to 3 knife-points) of chlorate of potash This is then covered with a glass plate and heated on a water bath for a few minutes. The glasses are then washed in hot water. Equal parts of soda, talcum, and sieved sawdust are now added with water enough to make a thick fluid mixture, which is now heated for one-half hour on the water bath, with frequent shaking of the contents of the beaker. The glasses are then washed again in hot water, to which a little hydrochloric acid has been added, especially, in case some calcium carbonate has been deposited on the glass Finally, wash in hot water or ethyl alcohol and dry with a soft cloth.

('94). van Hest, J J. Bakterienluftfilter und Bakterienluftfilterverschluss. Centralb. f. Bakt, Bd. XVI, 1894, pp. 435-447, also pp. 495-499, mit 11 figs.

('95). Knauss, K. Eine einfache Vorrichtung zum Abfüllen von je 10 ccm. Nährsubstanz. Centralb. f. Bakt, XVII Bd., 1895, pp. 878-879, with 1 fig.

('95). Banti, G Eine einfache Methode die Bakterien auf dem Agar und dem Blutserum zu isolieren. Centralb. f. Bakt., XVII Bd., 1895, pp. 556-557.

Agar is slanted, in broad tubes (diam. 2-3 cm). The material containing the bacteria is diluted to the proper amount with sterile bouillon or water. The condensation water is then inoculated by needle or loop, and subsequently tilted over the whole surface of the agar and allowed to drain back again.

('95). Bleisch, Max. Ein Apparat zur Gewinnung klaren Agars ohne Filtration. Centralb. f. Bakt., XVII Bd, 1895, pp. 360-362.

('95). Lode, Alois. Eine automatische Abfüllburette für Nährlösungen und Heilserum. Centralb f. Bakt., XVIII Bd, 1895, pp. 53-54, with 3 figs.

('95). Brunner, Conrad. Notiz zur Methode der Isolierung von Bakterien auf Agarplatten im Reagensglase. Centralb. f. Bakt., XVIII Bd., 1895, p. 59.

('95). Bujwid, O. Bemerkungen über die Filtration bakterienhaltiger Flüssigkeiten. Centralb. f. Bakt, XVIII Bd., 1895, pp. 332-333

Considers the Chamberland a less fragile and safer filter than the Berkefeld.

('95). Abel, Rudolf. Ein Halter für Objektträger und Deckgläschen. Centralb. f. Bakt., XVIII Bd., pp. 782-783, with 1 fig.

('96). Melnikow-Raswedenkow, M. Ueber die Einstellung des d'Arsonvalschen Thermostaten. Centralb. f. Bakt., XIX Bd, 1896, pp. 709-712, with 1 fig.

('96). Czaplewski. Bakteriologische Notizen Centralb. f Bakt., Bd. XX, 1896, pp 307-313.

Sixteen notes on methods of work.

('96). Kretz, Richard. Eine handliche und leicht sterilisierbare Abfüllvorrichtung für Kulturflüssigkeiten. Centralb. f. Bakt., xix Bd., 1896, pp. 73-74, with 1 fig.

('97). van't Hoff, H. J. Eine schnellere und quantitativ bessere Methode der bakteriologischen Plattenzählung. Centralb. f. Bakt., xxi Bd., 1897, pp. 731-733, with 1 fig.

Pours a suitable dilution of the bacteria on surface of gelatin or agar, and spreads fluid as widely as possible.

('97). Bolley, H. L. An apparatus for the bacteriological sampling of well waters. Centralb. f. Bakt., xxii Bd., 1897, pp. 288-290, with 1 fig.

('97). Kasparek, Theodor. Ein Vacuum-Apparat zum Abdampfen von Kulturen mit Ehmann'scher Wasserheizung. Centralb. f. Bakt., xxii Bd., 1897, pp. 6-7, with 1 fig.

('97). Kischensky, D. Ein Verfahren zur schnellen mikroskopischen Untersuchung auf Bakterien in Deckglas- und Objektträger-Präparatem. Centralb. f. Bakt., xxi Bd., 1897, pp. 876-877.

('97). Smith, Theobald. Ueber Fehlerquellen bei Prüfung der Gas- und Säurebildung bei Bakterien und deren Vermeidung. Centralb. f. Bakt., xxii Bd., 1897, pp. 45-49

('97). Novy, F. G. Neue Apparate zum Filtrieren und zum Sterilisieren durch Dampf. Centralb. f. Bakt., xxii Bd., 1897, pp. 337-340, with 3 figs.

('97). Schuermayer, B. Eine Abänderung des automatischen Gasabschlusses beim Verlöschen der Flammen an Brütschränken. Centralb. f. Bakt., xxi Bd., 1897, pp. 400-401, with 1 fig.

('97). Robertson, Sigismund. Ueber Objektträger- und Deckglashalter. Centralb. f. Bakt., xxi Bd., 1897, pp. 589-591, with 2 figs.

('98). Piorkowski. Ein neuer heizbarer Färbetisch. Deutsch. med. Wochenschr., 1898, No 20 Rev. in Centralb. f. Bakt., xxiv Bd., 1898, pp. 902-903, 1 fig.

('98). Smith, Theobald. One of the conditions under which discontinuous sterilization may be ineffective. Journal of Experimental Medicine, vol iii, 1898. Rev. in Centralb. f. Bakt., xxvi Bd., 1899, p. 585.

('98). Novy, F. G. Ein neuer Thermoregulator Centralb. f. Bakt., xxiii Bd., 1898, pp. 1,054-1,056, with 2 figs.

Made by Greiner and Friedrichs, Stuetzerbach, Thuringia, Germany.

('98). Murrill, Paul. Ein wirksamer Gasdruckregulator. Centralb. f. Bakt., xxiii Bd., 1898, pp. 1,056-1,059, with 2 figs.

('99). Kern, Ferdinand Eine automatische Messpipette für keimfreie Flüssigkeiten. Centralb. f. Bakt., xxv Bd., 1899, pp. 75-77, with 1 fig.

('99). Novy, F. G Collodium Sacs. See his book entitled Laboratory work in bacteriology, pp. 496-501.

('00). Bulloch, William. A simple apparatus for obtaining plate cultures or surface growths of obligate anaërobes Centralb. f. Bakt., xxvii Bd., 1900, pp. 140-142, with 1 fig.

Author uses the unguentum resinæ of the British Pharmacopœia as a luteing material This consists of resin in powder 200 grams, or 8 ounces; yellow beeswax 200 grams, or 8 ounces, olive oil [illegible] grams, or 5 ounces, and lard 150 grams, or 6 ounces. Melt together with gentle heat the resin and wax, add the other ingredients, strain through muslin, and cool with stirring.

('00). Wright, James H. A simple method for anaërobic cultivation in fluid media. Centralb. f. Bakt., xxvii Bd., 1900, pp. 74-75, with 1 fig.

('00). Stewart, C. Balfour. Apparatus for heating cultures to separate spore-bearing microorganisms. Centralb. f. Bakt., xxvii Bd., 1900, pp. 366- 367, with 1 fig.

This is a modification of Meyer's hot-air bath.

('00). Petri, R. J. Eine einfache Vorrichtung zum Abfüllen der Nährgelatine. Centralb. f. Bakt., xxvii, Bd., 1900, pp. 525-526, 1 fig.

('00). Nuttall, George H. F. Ein Apparat zur Herstellung von Rollkulturen. Centralb. f. Bakt., xxvii Bd., 1900, pp. 605-609, with 2 figs.

('00). Piorkowski. Ein Apparat zur Ermittelung von Desinfektionswirkungen. Centralb. f. Bakt., xxvii Bd., 1900, pp 609-610, with 1 fig.

('00). Epstein, Stanislaus. Ein neuer Thermoregulator. Centralb. f. Bakt., xxviii Bd., 1900, pp. 503-504, with 1 fig.

('00). Petri, R. J. Ein neuer Reagenzglasständer für Kulturen. Centralb. f. Bakt., xxviii Bd., 1900, pp. 747-748, with 1 fig.

('00). Smith, R. Greig. The measurement of bacteria. Proceedings Linnean Soc., New South Wales for 1900, Sydney, 1901, vol. xxv, pp. 533-536. Three figures in text. Also a separate (issued Nov. 22, 1900).

('00). Petri, R. J. Neue verbesserte Gelatine-Schälchen (verbesserte Petri-Schälchen). Centralb. f. Bakt., xxviii Bd., 1900, pp. 79-82, with 3 text figs.

This Petri-dish has a cover of yellow brown glass, of such a form that when they are piled one above the other all are protected from the action of the violet rays of the spectrum. These are made in two forms by Paul Altmann, Berlin.

('00). Petri, R. J. Neue anaërobe Gelatine-Schälchen-Kultur (verbesserte Petri-Schälchen) Centralb. f. Bakt., xxviii Bd., 1900, pp. 196-199, with 2 text figs.

('00). Wright, J. H. A simple method of cultivating anaërobic bacteria. Jour. Boston Soc Med. Sci., vol. v, 1900, pp. 114-115.

The plug is pushed part way down the test-tube containing the culture The cotton is then partly saturated with strong pyrogallic acid water (equal bulks of water and acid). Sodium hydrate solution (1 NaOH, 2 water) is then pipetted on, and the tube immediately plugged airtight with a soft rubber stopper The media should contain 1 per cent glucose. It should be fresh, and its reaction not more acid than + 15. It may be used for fluids, or roll cultures, and other forms.

('02). Hill, Hibbert Winslow. "Hanging block" preparations for the microscopic observation of developing bacteria. Journal of Med. Research, Boston, vol. vii, 1902 (new ser., vol. ii), pp. 202-212, 3 figs. Also a separate.

('02). Wherry, Wm. B. Experiments on the permeability of the Berkefeld filter and the Pasteur-Chamberland bougie to bacteria of small size. Jour. of Med. Research, vol. viii (n. s., vol. iii), 1902, pp. 322-328, 1 fig.

('02). Kellerman, Karl. A method for fixing and sectioning bacterial colonies, fungous mycelium, etc Jour. App. Micro., vol. v, 1902, p 1,080. Also a separate.

('02). Kellerman, Karl F. An improved method for making collodion tubes for dialyzing. Jour. App. Micro., vol v, 1902, p. 2,038.

('02). Carnot, Paul, et Garnier, Marcel. Sur la technique des cultures en tubes de sable. Paris, C. R. soc. biol., T. liv, 1902, pp. 748-750.

('02). Carnot, Paul, et Garnier, Marcel. De l'emploi des tubes de sable comme méthode générale d'etude, d'isolement et de sélection des microorganismes mobiles. Paris, C. R. soc. biol, T. liv, 1902, pp 860-863.

('02). Regaud, Cl. Nouveau bain de paraffine à chauffage et régulation électriques. J. anat. physiol, Paris, T. xxxviii, 1902, pp. 193-214, av. fig.

('02). Grijns, G. Eine einfache Vorrichtung, um zu verhindern, dass beim Gebrauch des Brütapparates für konstante niedrige Temperatur, System Lautenschläger (Katalog No 60, No 117), wenn das Eis im Behalter ausgeht, das ungekuhlte Wasser in den kalten Schrank fliesst. Centralb f. Bakt., Abt. 1, Bd. xxxi, Originale, 1902, pp. 430-432.

('02). Harris, Norman MacLeod. Concerning an improved method of making collodium sacs. Centralb. f. Bakt., Abt. 1, Bd. xxxii, Originale, 1902, pp. 74-80.

Dr. Harris makes his sacs around gelatin capsules.

('03). Gorsline, Charles S. On the preparation and use of collodium sacs. Vaughan Quarter Century Book, 1903, pp. 390-394. Ann Arbor, Mich., George Wahr.

('03). Winslow, C. E. A., and Nibecker, C. P. The significance of bacteriological methods in sanitary water analysis. Technology Quarterly, vol xvi, 1903, pp. 227-239. Also a separate.

('04). Remlinger, P. Le passage du virus rabique à travers les filtres. 2e mém. Ann de l'Inst. Pasteur, T. xviii, 1904, pp. 150-164.

('04). Report of the [English] committee appointed to consider the standardisation of methods for the bacterioscopic examination of water. Journal of State Medicine, August, 1904. See also Chemical News, vol. xc, Oct. 7, 1904, pp. 177-179.

Distilled-water agar and distilled-water gelatin are recommended for use along with nutrient agar and gelatin. In the search for *B. coli* the committee recommends either the glucose-formate broth of Pakes or the bile-salt broth of MacConkey.

XVIII. Special Means of Differentiating Bacteria.

('84). Gram. See xiv.

('87). von Rozsahegyi, A. Ueber das Züchten von Bakterien in gefärbter Nahrgelatine. Centralb. f. Bakt., ii Bd., 1887, No. 14, pp. 418-424

Author added various substances to his culture media, e g. fuchsin, methylen blue, gentian violet, vesuvin, etc. Different bacteria behave very differently as respects growth, absorption of pigment, and change of color in the pigment. Organisms may be differentiated in this way.

('88). Bujwid, O Neue Methode zum Diagnosticiren und Isoliren der Cholerabakterien. Centralb. f. Bakt., 1888, iv Bd., pp. 494-496.

In 24 hours, in 2 per cent feebly alkaline peptone solution at 37° C., the cholera organism gives a fine purple-red color on adding HCl. On longer cultivation other organisms give the same reaction. This depends on the formation of indol and a trace of nitrite.

('90). Petruschky, Johannes. Die Farbenreaktion bakterieller Stoffwechselprodukte auf Lackmus als Beitrag zur Charakteristik und als Mittel zur Unterscheidung von Bakterienarten. Centralb. f. Bakt., vii Bd., 1890, pp. 1-8 and 49-53

('92). Bujwid, Odo. Eine neue biologische Reaktion für die Cholerabakterien. Centralb. f. Bakt., xii Bd, 1892, pp. 595-596.

('92). Beyerinck, M. W. Notiz uber die Cholerarothreaktion. Centralb. f. Bakt., xii Bd., 1892, pp 715-718.

('93). Schild. Formalin zur Diagnose des Typhusbacillus. Centralb. f. Bakt., Bd. xiv, 1893, pp. 717-718

The typhoid organism will not grow in bouillon containing as little formalin as 1:15,000. B. coli develops vigorously with as much formalin as 1:3000.

('93). Mathews, Albert P. On Wurtz's Method for the Differentiation of Bacillus typhi abdominalis from Bacillus coli communis, and its application to the examination of contaminated drinking water. Technology Quarterly, vol. vi, 1893, pp. 241-251.

Litmus lactose gelatin or agar is reddened by B. coli and is unchanged or made deeper blue by B. typhosus. Author tests suspicious water by poured plates. After 11 hours at 37.5° C all blue colonies resembling typhoid are tested further, i e., in milk, gelatin, bouillon, nitrate solution, and sugar media.

('93). Gorini, Konstantin. Anmerkung über die Cholerarotreaktion. Centralb. f Bakt, Bd. xiii, 1893, pp. 790-792.

A good peptone for this purpose must be white, without odor, entirely soluble in water, especially on warming. The water solution must be clear, colorless, neutral or slightly alkaline, foamy on shaking It must give a violet reaction with Fehling's solution, which does not change on boiling. It must give no nitrite reaction with Griesz reagent, and finally must give, after about 5 minutes with Diphenylamine, a faint but distinct narrow, clear blue ring.

('94). Schneider, Paul. Die Bedeutung der Bakterienfarbstoffe fur die Unterscheidung der Arten. (Inaug. Diss) 8vo, 46 pp., 2 Taf., Basel, 1894 Rev in Centralb. f. Bakt., Bd. xvi, 1894, p. 633. See also Arbeiten a. d. bakt. Institut Karlsruhe 1, 1894.

('94). Marpmann. Zur Unterscheidung des Bacillus typhi abdominalis vom Bacillus coli commune. Centralb. f Bakt., Bd. xvi, 1894, pp. 817-820.

Uses various "chromo-agars" for differentiating bacteria, i. e., agar with fuchsin, malachit green, nigrosin, and indolin. Sodium bisulphit must be used with the the first two.

('94). Lunkewicz, M. Eine Farbenreaktion auf die salpetrige Säure der Kulturen der Cholerabacillen und einiger anderer Bakterien. Centralb f. Bakt., Bd. xvi, 1894, pp. 945-949.

Describes the preparations of the Griess-Ilosvay reagent.

('94). Abel, Rudolf. Ueber die Brauchbarkeit der von Schild angegebenen Formalinprobe zur Differential-Diagnose des Typhus bacillus. Centralb. f. Bakt, Bd. xvi, 1894, pp. 1,041-1,046.

Finds some bacteria more tolerant of formalin than was stated by Schild It is not a satisfactory method for separating typhoid bacilli from B. coli.

('95). MACKENSIE, J. J. Opening discussion on "What new methods can be suggested for the separation of bacteria into groups, and for the identification of species." Jour. Am. Pub. Health Asso., Oct., 1895, Ann. vol. XX, pp 419-431.

Notes on synthetic media.

('97). STODDART. See XVI.

('97). HISS. See XVI.

('98). HOUSTON, A. C. Note on four micro-organisms isolated from the mud of the river Thames, which resemble Bacillus typhosus Centralb. f. Bakt., XXIV Bd., 1898, pp. 518-525, colored diagram.

('98). FERMI. See XXXVI

('98). ROTHBERGER, C. JULIUS. Differential diagnostische Untersuchungen mit gefärbten Nährböden. Centralb. f. Bakt., XXIV Bd., 1898, pp. 513-518.

With neutral red (Toluidin red) in agar B. coli is said to cause a clearing of the color and a very decided fluorescence. B. typhi leaves the medium unchanged. The best method is said to be to add 3 to 4 drops of a concentrated water solution to 10 cc. of fluid agar, and then ½ cc. of a 24-hour old bouillon culture. In agar stained with safranin, B. coli reduces the color; B. typhi does not. Dead cultures of B. coli (cultures heated 2 hours at 78° C.) did not produce these changes.

('98). PACINOTTI, G., AND MUNIECKI, J. L'albume d'uovo colorito in verde-cupo dal caffè crudo, come mezzo diagnostico di sviluppi batterici. Gazz. degli ospedali e della cliniche, 1898, No. 31. Rev. in Centralb. f. Bakt., XXV Bd., 1899, p. 257.

('99.) ROTHBERGER, C. JULIUS. Differential diagnostische Untersuchungen mit gefärbten Nährböden. II, Mitteilung. Centralb. f. Bakt., XXV Bd., 1899, pp. 15-17 and 69-75.

Author tested the effect of various bacteria on 35 anilin dyes, 13 of which proved useful in agar media. The loss of color in methylen blue, safranin, Toluidin blue, Orseille extract and indigo carmin is due to reduction processes. In case of the indigo carmin, the blue was first changed to dark green.

('00). SCHEFFLER, W. Das Neutralrot als Hilfsmittel zur Diagnose des Bacterium coli. Centralb. f. Bakt., XXVIII Bd., 1900, pp. 199-205.

Author says B. coli in neutral red, grape sugar agar gives regularly in 24 to 48 hours a beautiful green fluorescence. He uses: fluid agar, 100 cc., grape sugar, 0.3 gram; concentrated watery solution of neutral red, 1cc.

('01). HOELSCHER, WALTER. Ueber die Differenz der histologischen Wirkung von Tuberkelbacillen und anderen diesen ähnlichen säurefesten Bacillen (Grasbacillus II Moeller, Butterbacillus Petri-Rabinowitsch, Thimotheebacillus Möller). Münchener med. Wochenschr., Bd. XLVIII, 1901, pp. 1,483-1,484.

('02). HISS, PHILLIP HANSON, JR. New and simple media for the differentiation of the colonies of typhoid, colon, and allied bacilli. The Journal of Medical Research, June, 1902, vol. VIII, pp. 148-167. Also a separate. 2 plates.

Author describes a simple solid medium in which buried colonies of the typhoid bacillus send out thread-formed radiations, while those of B. coli, etc., do not bear any fringing threads This medium, used in Petri-dish poured plates, consists of distilled water 1,000, agar 15, gelatin 15, dextrose 10, Liebig's extract meat 5, sodium chloride 5.

('02). FITZ GERALD, MABEL PUREFOY, AND DREYER, GEORGES. The unreliability of the neutral red method, as generally employed for the differentiation of B. typhosus and B coli. Festsksrift ved Indvielsen af Statens Seruminstitut. Kjöbenhavn, 1902, pp. 1-39.

('02). BUXTON, B. H. A comparative study of the bacilli intermediate between B. coli communis and B. typhosus. Jour. Med. Res. III (n. s.), pp. 201-230, 3 plates.

Bibliography of 16 titles.

('03). GAGE, STEPHEN DEM., AND PHELPS, EARLE B. Notes on B. coli and allied forms, with special reference to the neutral-red reaction. Proceedings of the Thirtieth Annual Meeting, American Public Health Assn., New Orleans, La., Dec., 1902. Pub. Columbus, Ohio, 1903, vol XXVIII, pp. 402-412. Also a separate, pp. 11.

('03). SCHUEDER. Zum Nachweis der Typhusbakterien im Wasser. Zeitschr. f. Hyg., Bd. XLII, 1903, pp. 317-326.

Describes differential methods.

('04). STOKES, WILLIAM ROYAL. A simple test for the routine detection of the colon bacillus in drinking water. Jour. of Infectious Diseases, vol. I, 1904, pp. 341-347. 1 plate.

Neutral red reaction.

XIX. Aerobism, Anaerobism.

(See also various citations under XX.)

('61). PASTEUR, LOUIS. Animalcules infusoires vivant sans gaz oxygène libre et déterminant des fermentations.—C. R. des sé. de l'Acad. des sci., Paris, 1861, T. LII, pp. 344-347.

('63). PASTEUR, LOUIS. Nouvel exemple de fermentation déterminée par des animalcules infusoires pouvant vivre sans oxygène libre, et en dehors de tout contact avec l'air de l'atmosphère.—C. R., des sé. de l'Acad. des sci., Paris, 1863, T. LVI, pp. 416-421.

('63). PASTEUR, LOUIS. Recherches sur la putréfaction. C. R. des sé. de l'Acad. des sci., Paris, 1863, T. LVI, pp. 1,189-1,194.

"Je propose avec toute sorte de scrupules ces mots nouveaux *aerobies* et *anaerobes* pour indiquer l'existence de deux classes d'êtres inferieures, les uns incapable de vivre en dehors de la présence du gaz oxygène libre, les autres pouvant se multiplier à l'infini en dehors du contact de ce gaz."

('80). BUCHNER. Ueber die Lebensfähigkeit der Spaltpilze bei fehlenden Sauerstoff, 1880.

Not seen.

('85). HESSE, W. UND R. Ueber Zuchtung der Bacillen des malignen Oedems. Deutsche med. Wochenschrift, 1885, 11 Jahrg., pp. 214-215.

Describes a method of cultivating anaerobic organisms by sowing them in deep masses of solid media.

('86). LIBORIUS, PAUL. Beiträge zur Kentniss d. Sauerstoffbedürfnisses der Bakterien. Zeitschr. f. Hyg., Bd. I, 1886, pp. 115-177.

According to review by Zimmerman in Bot. Centralb. Bd. XXVIII, 1886, p. 108, author found but little more oxygen in layers of agar buried 3 cm. and over under additional agar than in vessels in which air was expelled by hydrogen, etc. He used "Indigotinloesung" as a test.

('87). Roux, E. Sur la culture des microbes anaérobies. Ann. de l'Inst. Pasteur, Bd. I, 1887, pp. 49-62.

('88). Buchner, Hans. Eine neue Methode zur Kultur anaërober Mikroorganismen. Centralb. f. Bakt., 1888, IV Bd., pp. 149-151.

The pyrogallic acid method. If 1 gram pyrogallol and 10 cc. 10 per cent potash water are used for each 100 cc. air space, all oxygen is removed in 24 hours at 37°C. The use of this mixture liberates small quantities of carbonic oxide, which is less in quantity in proportion as the absorption of the oxygen is rapid. Buchner observed no injurious influence of this gas on the bacteria with which he experimented.

('88). Fraenkel, Carl. Ueber die Kultur anaërober Mikroorganismen. Centralb. f. Bakt., 1888, III Bd., pp. 735-740 and 763-768 1 fig.

('89). Fraenkel. See XXXVIII.

('89). Frankland, Percy. On the influence of carbonic anhydride or other gases on the development of micro-organisms. Proc. Roy. Soc., vol. XLV, 1889, pp. 292-301. See also Zeitsch. f. Hygiene, Bd. VI, 1889, pp. 13-22.

The other gases tested were hydrogen, carbonic oxide, and nitrous oxide. Hydrogen had the least deleterious effect on the organisms tested, which were B. pyocyaneus, Koch's comma bacillus, and Finkler's spirillum.

('90). Smith. See XX.

('90). Popoff. See XLVIII.

('92). Ogata, M. Einfache Bakterienkultur mit verschiedenen Gasen. Centralb. f. Bakt., XI Bd., 1892, pp. 621-623, mit 1 fig.

Ogata's method, which is essentially that previously described by Heim, consists in softening the upper part of a test tube in the flame and drawing it out so that the tube consists of two normal portions connected by a narrow isthmus. This part should be just below the cotton plug, that is, in the upper one-third of the tube. A piece of glass tubing is now plugged at one end with sterile cotton, and is drawn out into a capillary tube, which must be long enough to reach down into the bottom of the culture medium. The broken end of this tube is now touched to the desired culture and passed into the test tube. The cotton-plugged end of the glass tube is now attached to a rubber tube connected with the gas apparatus. After the gas has bubbled through the medium for a sufficient time, the capillary tube is removed, and the test tube is immediately sealed in the open flame by a further constriction of the isthmus and a complete removal of the upper part of the test tube.

Fluid cultures may also be made in such tubes by preparing the isthmus before the tubes are filled with the medium.

('92). Heim, L. Zur Originalmittheilung von Ogata: "Einfache Bakterienkultur mit verschiedenen Gasen." Bd. XI, p. 621. Centralb. f. Bakt., XI, 1892, p. 800.

('92). van Senus, A. H. C. Zur Kenntniss der Kultur anaërober Bakterien. Centralb. f. Bakt., XII Bd., 1892, pp. 144-145.

('93). Novy, F. G. Die Kultur anaërober Bakterien. Centralb. f. Bakt., Bd. XIV, 1893, pp. 581-600, 2 figs.

The "Novy jars" are here figured and described, and 10 references to literature are given at the end of the article.

('93). Beyerinck, M. W. Ueber Atmungsfiguren beweglicher Bakterien. Centralb. f. Bakt., Bd. XIV, 1893, pp. 827-845, mit 1 Tafel.

('93). Smith. See XX.

('94). Nicolaier. Bemerkung zu der Arbeit von Prof. F. G. Novy: "Die Kultur anaërober Bakterien" (Centralb. f. Bakt., Bd. XIV, 1893). Centralb. f. Bakt., Bd. XV, 1894, p. 227.

('94). Smith, Theobald. Further observations on the fermentation tube, with special reference to anaërobiosis, reduction, and gas production (abstract). Proc. Am. Ass. A. Sci., 42, Madison Meeting, 1893, Salem, 1894, p. 261.

('94). Engelmann, Th. W. Die Erscheinungsweise der Sauerstoffausscheidung chromophyllhaltiger Zellen im Licht bei Anwendung der Bacterienmethode. Verhand. d. Kon. Akad. van Wetenschappen te Amsterdam (2 te sectie, deel III; No. 11, 1894), pp. 10, IV, with a folded plate, also a reprint, 17 pp. Gives a bibliography of 61 titles. See also Onderzoekingen Physiol. Laborat. Utrecht, IV Reeks, III deel., 1895.

('94). Lubinski, Wsewolod. Zur Methodik der Kultur anaërober Bakterien. Centralb. f. Bakt., Bd. XVI, 1894, pp. 20-25, mit 4 figs.

('94). Novy, F. G. Die Plattenkultur anaërober Bakterien. Centralb. f. Bakt., Bd. XVI, 1894, pp. 566-571, with 3 figs.

('94). Arens. Eine Methode zur Plattenkultur der Anaeroben. Centralb. f. Bakt., Bd. XV, 1894, pp. 15-17.

The author makes his cultures in ordinary exsiccators having ground glass covers.

('95). Schmidt, Ad. Eine einfache Methode zur Züchtung anaërober Kulturen in flüssigen Nährböden. Centralb. f. Bakt., XVII Bd., 1895, pp. 460-461, 1 fig.

('95). Braatz, Egbert. Einiges über die Anaërobiose. Centralb. f. Bakt., XVII Bd., 1895, pp. 737-742, with 1 fig.

('95). Kedrowski, W. Ueber die Bedingungen, unter welchen anaërobe Bakterien auch bei Gegenwart von Sauerstoff existiren können. Zeitschr. f. Hyg., Bd. XX, 1895, pp. 358-375.

They do this best when mixed with aerobes, but the absorption of the oxygen is not so important as Pasteur supposed. The author believes that the aerobes excrete some special substance favorable to the growth of the anaerobes. This substance he did not determine.

('95). Gerstner, R. Beiträge zur Kentniss obligat-anaërober Bakterienarten. Arbeiten aus dem Bakteriologischen Institut der Technischen Hochschule zu Carlsruhe, Bd. I, 1895. H. 2, pp. 148-183, with 2 Taf.

('96). Kasparek, Theodor. Ein einfacher Luftabschluss flüssiger Nährböden beim Kultivieren anaërober Bakterien. Centralb. f. Bakt., XX Bd., 1896, pp. 536-537, 2 figs.

('96). Durham, Herbert E. On a self-acting means of cultivating anaerobic microbes. Jour. Path. and Bact., vol. III, 1896, pp. 231-236.

('97). Beck, M. Zur Züchtung anaërober Kulturen. Centralb. f. Bakt., XXII Bd., 1897, pp. 343-345, with 2 figs.

('98). Scholtz, W. Ueber das Wachstum anaërober Bakterien bei ungehindertem Luftzutritt. Zeitschr. f. Hyg., Bd. XXVII, 1898, pp. 132-142. Review in Centralb. f. Bakt., XXIV Bd., 1898, p. 932.

('98). Ferran, J. Ueber das aërobische Verhalten des Tetanusbacillus. Centralb. f. Bakt., XXIV Bd., 1898, pp. 28-29.

Thinks his experiments show tetanus bacillus is not a strict anaerobe, but only a facultative anaerobe.

('98). KLEIN, ALEX. Ein Apparat zur bequemen Herstellung von anaëroben Plattenkulturen. Centralb. f. Bakt., XXIV Bd., 1898, pp. 967-971, 2 figs.

The reviewer has not had that trouble with Novy's apparatus complained of by Mr. Klein. The author's siphon arrangement for mixing the potash water with the pyrogallol after exhaustion of the air appears to be very good.

('98). FERRAN, J. Ueber die Verwendung des Acetylens bei der Kultur anaërober Bakterien. Centralb. f. Bakt., XXIV Bd., 1898, p. 29.

('98). ZUPNIK, LEO. Ueber eine neue Methode anaërober Züchtung. Centralb. f. Bakt., XXIV Bd., 1898, pp. 267-270, with 1 fig.

('98). OPRESCU. Zur Technik der Anaërobenkultur. Hyg. Rundschau, 1898, No. 2. Rev. in Centralb. f. Bakt., XXIII Bd., 1898, p. 668.

('98). UCKE, ALEXANDER. Ein Beitrag zur Kenntnis der Anaëroben. Centralb. f. Bakt., XXIII Bd., 1898, pp. 996-1,001.

('98). TRENKMANN. Das Wachstum der anaëroben Bakterien. Centralb. f. Bakt., XXIII Bd., 1898, pp. 1,038-1,043 and 1,087-1,090.

('98). MARPMANN. Eine neue Methode zur Herstellung von anaëroben Rollglaskulturen mit Gelatine oder Agar. Centralb. f. Bakt., XXIII Bd., 1898, pp. 1,090-1,091.

('98). EPSTEIN, STANISLAUS. Apparat zur Kultur anaërober Bakterien. Centralb. f. Bakt., XXIV Bd., 1898, pp. 266-267, 1 fig.

('99). KABRHEL, GUSTAV. Zur Frage der Züchtung anaërober Bakterien. Centralb. f. Bakt., XXV Bd., 1899, pp. 555-561, with 1 fig.

As an oxygen indicator author uses methylene blue in sugar gelatin.

('99). SMITH, THEOBALD. Some devices for the cultivation of anaerobic bacteria in fluid media without the use of inert gases. Jour. Bost Soc. Med Sci., 1899, pp. 340-343. Also a separate, 4 pp.

('00). EPSTEIN, STANISLAUS Ein vereinfachtes Verfahren zur Zuchtung anaërober Bakterien in Doppelschalen. Centralb. f. Bakt., XXVIII Bd., 1900, p. 443, with 1 fig.

('00). PETRI. See XVII.

('00). KRAUSE. See XV.

('00). BULLOCK. See XVII.

('00). WRIGHT. See XVII

('02). OMÉLIANSKI, W. Ein einfacher Apparat zur Kultur von Anaëroben im Reagenzglase Centralb. f. Bakt., Abt. 2, Bd VIII, 1902, pp. 711-714.

XX. Fermentations, Gas-Formation, Enzymes, Etc.

(See also XIX and XLVIII.)

('57). PASTEUR, L. Mémoire sur le fermentation appelée lactique. C. R. des sé. de l'Acad. des sci., T. XLV, 1857, pp. 913-916.

('77) VINES, S. H. On the digestive ferment of Nepenthes. Journ. Linn. Soc. (Bot.), vol. XV, 1877, pp. 427-431.

('79). v. NAEGELI, CARL WILHELM. Theorie der Gärung. Ein Beitrag zur Molecularphysiologie. München, 1879, pp. IV, 156.

('79). PRAZMOWSKI, A. Zur Entwicklungsgeschichte und Fermentwirkung einiger Bacterien-Arten. Vorläufige Mittheilung. Bot. Zeitung, Bd. XXXVII, No. 26, col. 409-424, 1879

('79). VAN TIEGHEM, P. E. L. Sur la fermentation de la cellulose.—Bull. de la Société Bot. de France, 1879, T. XXVI, pp. 25-30.

('82). MAYER, ADOLF. Die Lehre von den chemischen Fermenten oder Enzymologie. pp. VI, 124. Heidelberg, 1882.

('82). BÉCHAMP, A. Sur les microzymas comme cause de la décomposition de l'eau oxygénée par les tissus des animaux et des végétaux C. R. des sé. de l'Acad. des sci., Paris, T. XCIV, pp. 1,653-1,656.

('82). BERT, B., ET REGNARD, P. Action de l'eau oxygénée sur les matières organiques et les fermentations. C. R. des sé. de l'Acad. des sci., Paris, T. XCIV, pp. 1,383-1,386. Jour. de Pharm. et de Chimie, T. VI, 5 série, 1882, pp. 14-17.

('82). WORTMANN, J. Untersuchungen über das diastatische Ferment der Bacterien. Zeitschr. f. physiol. chem., Bd. VI, pp. 287-329, 1882. Also Jour. Chem. Soc., XLIV, pp. 930-938, 1883.

('82). MARCANO, V. Fermentation directe de la fécule. Mécanisme de cette métamorphose. C. R. des sé. de l'Acad. des Sci., Paris. T. XCV, 1882, pp. 856-859.

('83). BÉCHAMP, A. Les microzymas dans leurs rapports avec l'hétérogénie, l'histogénie, la physiologie et la pathologie, pp. XXXVIII, 992, with 3 pl. Paris, 1883.

Prior to 1883 Béchamp isolated from yeast an enzyme capable of inverting cane sugar. See pp. 69 et seq.

('83). VIGNA, A. Ueber Bakteriengährung des Glycerins. Ber. d. deutsch. chem. Gesells., XVI, pp. 1,438-1,439; Gazz. Chim. Ital., vol XIII, pp. 293-296, 1883.

('83). TAPPEINER, H. Ueber Cellulosegährungen Ber. d. deutsch. chem. Gesells., Bd. XVI, 1883, pp. 1,734-1,740.

('83). TAPPEINER, H. Ueber die Sumpfgasgährung im Schlamme der Teiche, Sümpfe und Kloaken. Ber. d. deutsch. chem. Gesells., Bd. XVI, 1883, pp. 1,740-1,744.

('86). HOPPE-SEYLER, FELIX. Ueber Gährung der Cellulose mit Bildung von Methan und Kohlensäure. Zeitschr. f. physiolog. Chemie., 1886, Bd. X, pp. 201-217 and 401-440.

('89). KRAMER, ERNST. Studien über schleimige Gährung. Sitzungsb. d. k. Ak. d. Wissenschaften. Math.-naturw. Classe, Bd. XCVIII, 1889, IV Hft., Abt II b. Chemie, pp. 358-396. Wien.

An interesting paper.

('89). FRANKLAND, PERCY F., AND FOX, J. J. On a pure fermentation of mannite and glycerine Proc. Royal Soc., Lond., 1889, vol XLVI, pp 345-357.

('89). CURTMAN, CHAS. O. Nachweis der Glycose durch Safranin. Pharmaceutische Rundschau, Bd. VII, 1889, p. 132.

('89). TISCHUTKIN, N. Die Rolle der Bakterien bei der Veranderung der Eiweissstoffe auf den Blattern von Pinguicula. Ber. d. deutsch. bot. Gessellsch., Bd. VII, 1889, pp 346-355.

('90) SCLAVO, ACHILLE, UND GOSIO, B. Ueber eine neue Garung der Stärke. Staz. sperim. agrar. ital., 1890, vol. XIX, p. 540.

('90). REINITZER, FR. Ueber die wahre Natur des Gummifermentes. Zeitschr. f. physiol. Chemie, Bd. XIV, 1890. pp. 453-470.

This author states that Wiesner's ferment, which changes cellulose into gum and slime, does not exist. Nickel, of Berlin, has come to the same conclusion independently. (See Bot. Centralb., 1890, Bd. XLIII, p. 118). The "orcin salzsaure" reaction of Reichl is a furfurol reaction.

('90). SMITH, THEOBALD. Das Gährungskölbchen in der Bakteriologie. Centralb. f. Bakt., VII Bd., 1890, pp. 502-506, mit 1 Abbildung.

('90). SMITH, THEOBALD. Einige Bemerkungen über Säure- und Alkalibildung bei Bakterien. Centralb. f. Bakt., Bd. VIII, 1890. pp. 389-391.

('90). FERMI, CLAUDIO. Die Leim und Fibrin lösenden und die diastätischen Fermente der Mikroorganismen. Centralb. f. Bakt., VII Bd., 1890, pp. 469-474.

('90). DUBOIS, R. Sur le prétendu pouvoir digestif du liquide de l'urne des Nepenthes. C. R. des sé. de l'Acad. des Sci. T. CXI, 1890, pp. 315-317.

Maintains that the observed results are not due to any pepsin-like body secreted by the plant, but to the action of intruding bacteria, and concludes that the Nepenthes are not carnivorous.

('91). HÉRY. Sur une fermentation visqueuse de l'encre —Annales de Micrographie, 1891, T. IV, pp. 13-21.

('91). FERMI, CLAUDIO. Weitere Untersuchungen über die tryptischen Enzyme der Mikroorganismen. Centralb. f. Bakt. X Bd, 1891, pp. 401-408.

('91). RITSERT. Bakteriologische Untersuchungen über das Schleimigwerden der Infusa. Ber. d. pharm. Gesellsch., 1 Bd, 1891. pp. 389-390. Rev. in Centralb. f. Bakt., 1892, XI, pp. 730-733.

('91). SUCHSLAND, E. Ueber Tabaksfermentationen. Ber. d. d. bot. Gesellsch., Bd. IX, 1891, pp. 79-81.

('91) BEYERINCK, M. W Verfahren zum Nachweise der Säureabsonderung bei Mikrobien. Centralb. f Bakt., IX Bd., 1891, pp. 781-786.

This method consists in using with gelatin or agar, for plate-cultures, etc, a solid opaque substance which is converted into a soluble, transparent substance in the presence of acids. For this purpose Beyerinck adds to the culture-medium a small amount of water in which a very finely divided carbonate of lime has been shaken up. This makes a white opaque plate. Colonies which secrete acids are soon surrounded by a transparent diffusion field. Other carbonates may be used, e.g, zinc carbonate. This method may also be used for the demonstration of alkali production.

('91). TISCHUTKIN, N. P. Ueber die Rolle der Mikroorganismen bei der Ernährung der insektenfressenden Pflanzen. Arbeit. d. St. Petersburg naturf. Gesell, vol. for 1890, Sect. d. Bot pp. 33-37, St. Petersburg, 1891. Abs in Bot. Centralb., Bd L, 1892, p. 304.

('92) CONN, H. W. Isolirung eines "Lab" fermentes aus Bakterienkulturen. Centralb. f. Bakt., Bd. XII, 1892, pp. 223-227.

('92?). FRANKLAND AND FREW. The fermentation of calcium glycerate by the Bacillus ethaceticus. Trans. Roy. Chem. Soc., 1891. Rev. in Centralb. f. Bakt., XII Bd., 1892, p. 724.

('92). FRANKLAND, P., AND MACGREGOR, J. Fermentation of arabinose with the Bacillus ethaceticus. Trans. Chem. Soc, 1892. Rev. in Centralb. f. Bakt., XII Bd., 1892, p. 725.

('92). FRANKLAND, P. F., AND FREW, W. A pure fermentation of mannitol and dulcitol. Trans. Chem. Soc., 1892, pp. 254-277.

('92). FRANKLAND, P. F., AND LUMSDEN, J. S. Decomposition of mannitol and dextrose by the Bacillus ethaceticus. Trans. Chem. Soc., 1892, pp. 432-444.

('92). FERMI, CLAUDIO. Beitrag zum Studium der von den Mikroorganismen abgesonderten diastätischen und Inversionsfermente. Centralb. f. Bakt., XII Bd., 1892, pp. 713-715.

Of 62 bacteria investigated, 20 were acid producers, 2 inverted cane sugar, 24 developed a proteolytic enzym, 20 a diastasic ferment.

('92). TISCHUTKIN, N. Ueber die Rolle der Microorganismen bei der Ernährung insektenfressender Pflanzen. Acta Horti Petropolitanie, Bd. XII, St. Petersburg, 1892, pp. 1-19. Abstract in Bot. Centralb., Bd. LIII, 1893, p. 322.

('92). FRANKLAND, P. F. Cantor lectures on recent contributions to the chemistry of bacteriology of the fermentation industries, gr. 8vo., 31 pp. London, W. S. Trounce, 1892.

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('93). HILDEBRANDT, H. Weiteres über hydrolytische Fermente, etc. Arch. f. path. Anat. u. Physiol., Bd. CXXXI, 1893, pp. 5-39.

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('81). Pasteur, Chamberland, et Roux. De l'atténuation des virus et de leur retour à la virulence. C. R. des sé. de l'Acad. des sci., T. xcii, 1881, pp. 429-435.

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('83). Chamberland et Roux. Sur l'atténuation de la bactéridie charbonneuse et de ses germes sous l'influence des substances antiseptiques. C. R. des sé. de l'Acad. des sci., T. xcvi, 1883, Paris, pp. 1,410-1,412.

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('89). Macé. See xxiii.

('93). d'Arsonval and Charrin. See xxxii.

('94). Roger. See xxxii.

('95). Klepzoff. See xxxiii.

('03). Fuhrmann, Franz. Ueber Virulenzsteigerung eines Stammes des Vibrio Cholerae asiaticae. Sitzungsber. d. kaiserl. Akad. d. Wissensch. Mathematisch-Naturwissensch. Klasse., Bd. cxii, Heft. viii, Abt. iii, 1903, pp. 267-284. With bibliography of 15 titles

XXIII. Pigments. Green Bacteria.

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('80). van Tieghem, Ph. Observations sur des bactériacées vertes, sur des phycochromacées blanches, et sur les affinités de ces deux familles. Bull. de la Soc. Bot. de France, Tome xxvii, 1880, pp. 174-179.

('82). Engelmann, Th. W. Zur Biologie der Schizomyceten. Bot. Zeitung, 40 Jahrg., 1882, col. 321-325 and 337-341.

Describes a green organism as Bacterium chlorinum. It is believed to contain chlorophyll

('87). Schottelius. See xv.

('87). Prove, Oskar. Micrococcus ochroleucus eine neue chromogene Spaltpilzform. Beitr. zur Biol. der Pflanzen., Bd. iv, Hft. 3, 1887, pp. 409-430, 1 plate.

('89). Macé. Sur la récupération de la vitalité des cultures de bactéries par passages sur certains milieux. Soc. des sc. de Nancy pour 1888, Series ii, T. ix, Fasc. xxii, pp. xxix-xxx and 79-83, Paris, 1889.

The medium used was potato. Bacteria which have lost their power to form pigment on agar or to infect frogs will often regain these functions if cultivated for a time on potato.

('89). Zopf, W. Ueber Pilzfarbstoffe. iv. Vorkommen einer Lipochroms bei Spaltpilzen. Bot. Zeitung, Bd xlvii, 1889, col 89-92

('89). Zopf, W. Ueber das mikrochemischen Verhalten von Fettfarbstoffen und Fettfarbstoffhaltigen Organen. Zeitschr. f. wissensch. Mikroskopie, 1889, Bd vi, pp. 172-177.

('89) Scheibenzuber, D. Ein Bacillus mit brauner Verfarbung der Gelatine. Allgemeinen Wiener medicinischen Zeitung, Jahrg. xxxiv, 1889, pp. 171-172, Wien, 1889. Also a separate, 7 pp.

This organism liquefies gelatin. It was isolated from spoiled eggs.

('90). Behr, P. Ueber eine nicht mehr farbstoffbildende Rasse des Bacillus der blauen Milch. Centralb f. Bakt., Bd. viii, 1890, pp. 485-487.

('90). Claessen, Heinr. Ueber einen indigoblauen Farbstoff erzeugenden Bacillus aus Wasser Centralb. f. Bakt., Bd. vii, 1890, pp. 13-17.

('91). Dangeard, P. A. Contribution à l'étude des Bactériacées vertes (Eubacillus gen. nov.). Le Botaniste, Ser. ii, 1891, fasc 4, pp. 151-160, avec. planche. Also C. R. des sé. de l'Acad. des sci., T. cxii, 1891, pp 251-253. Review in Centralb. f. Bakt., x Bd., 1891, pp. 745-747.

Dangeard found his green spore-bearing organism (Eubacillus multisporus) on the walls of a culture flask used for the growth of fresh-water algæ, where it made a felt of very long slender flexible filaments. He states that it contains chlorophyll distributed through the protoplasm The sporiferous filaments may be branched The spores are a distinct green. Its habitat is among fresh-water algæ in the vicinity of Caen, France.

To the second section of his genus he would add the following forms, described by Klein: Bacillus de Baryanus, B. Solmsii, B. Peroniella, B. macrosporus, and B limosus, all of which are sporiferous, the spores being blue-green. These spores are all said to be impregnated with chlorophyll.

('91) Slater, Charles. On a red pigment forming organism, Bacillus corallinus. The Quarterly Jour. of Microsc. Sci., 1891, vol. 32, n. s., No. cxxvii, pp. 409-416, 1 plate.

The author says budding and branched forms occur in liquid media.

('91). Beyerinck, M. W. La biologie d'une bactérie pigmentaire. Archives néerlandaises, T. xxv, 1891, Livr., 314. See also Die Lebensgeschichte einer Pigmentbakterie. Bot. Zeitung. 1891, Nos. 43, 45, 46, and 47, columns 705-712, 741-752, 757-770, and 773-781, with 1 plate.

This paper describes the morphology and biology of Bacillus cyaneofuscus, which was first found causing damage to the glue of a gelatin factory and afterwards in other places. "This microbe is also the cause, or at least one of the causes, of a deterioration quite frequent in Holland cheeses, especially those known as 'Edam,' a change which, under the name of 'blueing,' is much dreaded by makers of these products."

This organism belongs to the chromoparous group of pigment-forming bacteria, i. e., it is itself colorless, but excretes the pigment. The first form of the pigment is a blue-green substance, which, under the microscope, appears as solid-blue spherites, mixed in with the bacteria.

('91). Overbeck, A. Zur Kenntniss der Fettfarbstoff-Produktion bei Spaltpilzen. Nova Acta d. K. Leop. Carol. Deutschen Akad. d. Naturf., Bd. lv, No 7, pp. 399-416. 1 plate. Halle, 1891. Also a separate.

('91) Beijerinck, M. W. De Levensgeschiedenis eener Pigmentbacterie. Versl. en Mededeel. der koninkl. Akad. van Wetensch. Afdeeling Naturkunde, 3 Reeks, 8 Deel, 1891, pp (307)-(315).

Paper deals with Bacillus cyaneofuscus.

('91). Gessard, C. Des races du bacille pyocyanique. Ann. de l'Inst. Pasteur, T. v, 1891, pp. 65-78

Organism produces two pigments (a) Pyocyanin (b) fluorescine, and probably a third.

('91). Zopf, W Ueber Ausscheidung von Fettfarbstoffen (Lipochromen) seitens gewisser Spaltpilze. Ber. d. deutsch. bot Gesellsch., Bd. ix, pp 22-28, 1891.

('92). Gessard, C. Sur la fonction fluorescigène des microbes. Ann. de l'Inst. Pasteur. T. vi, 1892, pp. 801-823.

('92). Charrin et Physalix. See xv.

('92). Rohrer. Ueber die Pigmentbildung des Bacillus pyocyaneus Centralb. f. Bakt., xi Bd., 1892, pp 327-335.

('92). Okada, K. Ueber einen rothen Farbstoff erzeugenden Bacillus (Bacillus rubellus) aus Fussbodenstaub. Centralb. f. Bakt, xi Bd, 1892, pp. 1-4, mit 1 colored Taf.

('93) Ernst, Harold C. The Bacillus pyocyaneus pericarditidis. The American Journal of the Medical Sciences, 1893, October, No. 258, pp. 396-402. Rev. in Centralb. f. Bakt., Bd. xv, 1894, p. 559

This organism is motile by means of a polar flagellum, and the writer has changed the name to Bacterium pericarditidis (Ernst). It produces no pigment soluble in chloroform.

('93) Voges, O. Ueber einige im Wasser vorkommende Pigmentbakterien. Centralb f. Bakt, Bd. xiv, 1893, pp. 301-315.

An account of Bacillus cœrulens and other blue or violet pigment-forming organisms.

('93). d'Arsonval and Charrin. See xxxii.

('94). Bordoni-Uffreduzzi. See xlvii.

('94). Teissier, P. J. Etude des propriétés chromogènes permanentes ou facultatives de certains microbes pathogènes ou saprophytes cultivés sur l'albumine de l'œuf coagulé. Arch. de méd. expérim. et d'anatomie pathologique, Tome vi, 1894. No. 2, pp. 315-327

('94). Guignard et Sauvageau. Sur un nouveau microbe chromogène, le Bacillus chlororaphis. C. R. des sé. et mém. de la Soc. de Biol., Paris, sé. x, T. i, 22 Dec., 1894, pp. 841-843.

('95). Schneider, Paul. Die Bedeutung der Bacterienfärbstoffe für die Unterscheidung der Arten. Arbeiten aus dem bakt. Institut der Technischen Hochschule zu Karlsruhe. Bd. i, Hft. 2, 1895, pp. 201-232. 1 fig. and 1 Tafel.

Conclusions (1) Distinguishable in part by diverse behavior in solvents. (2) The same organism, under like conditions, always produces the same pigment. (3) Two sorts may produce the same pigment. (4) Most of those sorts which produce apparently the same pigment, and are much alike in other ways, may be distinguished easily by the different reactions of the pigments.

('95). Dangeard, P. A. Observations sur le groupe des bactéries vertes. Ann. de micrographie, 1895, T. vii, pp. 67-69.

Considers Bact. viride, van Tieghem, to belong to the algæ.

('95). Thumm, K. Beitrage zur Biologie der fluorescirenden Bakterien. Arbeiten aus dem Bakt. Inst. der Technischen Hochschule zu Carlsruhe, Bd. I, 1895, pp. 291-377.

The following summary of the most important results is introduced in extenso, owing to the difficulty of procuring the original paper:

1. All fluorescent bacteria show in alkaline gelatin, first a sky-blue, later a moss-green fluorescence, and with the latter a yellowing of the substratum. Old cultures, with the exception of those of Bacillus fluorescens putidus, are orange-red, with a dark-green fluorescence.

2. All these colors are due to one yellow pigment, a concentrated watery solution of which is orange yellow, a dilute one yellow. Both fluids are blue fluorescent, but, upon the addition of an alkali, become, according to the concentration, dark-green or moss-green fluorescent.

3 All species produce the same pigment.

4. All are alkali formers. The production of ammonia in many species is considerable, and it is due to the presence of this alkali that the blue fluorescence gives place to the green.

5. The view of Naegeli, Ledderhose, and Kunz of the formation of a leuco-pigment, and their attempt to trace back the different colorings to oxidation phenomena, has not been confirmed.

6. Bacillus pyocyaneus Ernst, contrary to the view of the other investigators, forms only one pigment.

7. In potato cultures and on acid gelatin, the same coloring matter is produced as in alkaline media. The green fluorescence is, in every case, caused by the action of the ammonia.

8. The α and β forms of Bacillus pyocyaneus differ only by the amount of ammonia produced not by a difference in the pigment formed. The α form is a good, the β form a poor alkali producer When ammonia is added to a culture of the β form, it resembles a culture of the α form.

9. When an acid producer and a fluorescent form are grown together in the same culture, the yellow pigment appears normally, but there is no fluorescence.

10. All species have the power of oxidizing grape sugar with the production of an acid. The ammonia formed later neutralizes this.

11. The addition of sodium formate to the ordinary nutrient gelatin causes an increased ammonia production.

12. In hydrochinon gelatin all species produce a brownish-red color, due to the action of ammonia on the hydrochinon. This may, under certain circumstances, be used as a test for ammonia.

13. The behavior of the different species in media containing different organic substances is so characteristic that it may serve as a valuable means of differentiating related species. Ammonium succinate, or asparagin, affords good nourishment for all species.

14. According to the source of the carbon or nitrogen. the same organism is an abundant or scanty alkali producer; e. g., Bacillus pyocyaneus Ernst is a poor alkali former in nutrient gelatin, but a good one in ammonium succinate.

15. Each organism shows manifestations of life only when it comes in contact with the oxygen of the air. Hence, only there do we find pigment and formation of ammonia.

16. For the formation of the pigment, calcium chloride is entirely unessential, but magnesium sulfate and potassium phosphate are of the greatest importance. Gessard's view, that only phosphoric acid is absolutely necessary, is erroneous, nor may it ever be concluded that absence of fluorescence it due to absence of phosfates.

17. The substitution of calcium for magnesium, and vice versa, does not influence the development of the different species, but in the formation of the pigment, calcium can not take the place of magnesium.

18. The blue color of a fluid culture of Bacillus pyocyaneus, in the absence of phosphoric acid, is never caused by pyocyanin, as Gessard assumed, but is referable only to refraction phenomena.

19. The least amount of potassium phosfate or magnesium sulfate is sufficient for the formation of the fluorescent pigment. Where, in such nutrient solutions, mostly blue fluorescence is to be observed, there has been a decreased amount of ammonia produced because of the small supply of nutrient salts in the solution.

20. Bacterium syncyaneum has the power of forming two pigments, a fluorescent and a steel-blue one. The former agrees with that of the other fluorescent species. The latter varies from steel-blue to brownish black, according to the reaction of the medium.

21. The fluorescence of the β form of Bacterium syncyaneum may be produced by cultivating it in ammonium lactate and transferring to nutrient gelatin.

('96). Biel, Wilhelm. Ueber einen schwarzes Pigmentbildenden Kartoffelbacillus. Centralb. f. Bakt., 2 Abt., Bd. II, 1896, pp. 137-140.

('96). Scheurlen. Geschichtliche und experimentelle Studien über den Prodigiosus. Archiv. f. Hyg., 1896, Bd. XXVI, pp. 1-31.

('96). Gorini, C. Ueber die schwarzen pigmentbildenden Bakterien. Centralb. f. Bakt., I Abt., Bd. XX, 1896, p. 94.

('97). Ewart, A. J. Bacteria with assimilatory pigments, found in the tropics. Annals of Botany, vol. XI, 1897, pp. 486-487.

Author found seven greenish bacteria in water at Buitenzorg, Java, showing a faint evolution of oxygen when exposed to light. These were B. chlorinum and Streptococcus varians, two forms closely resembling van Tieghem's B. virens and Bact. viride; another large bacillus, somewhat resembling van Tieghem's B. virens, and two Spirilla. The red Bacterium photometricum, which is common in Java, gives, on treatment with hot alcohol and extraction with benzine, a green dye which seems to be identical with chlorophyll.

('97). Thiry, G. Contribution à l'étude du polychromisme bactérien. Bacille et Cladothrix polychromes; cristaux colorés. Arch. de physiol., 1897, No. 2, pp. 284-288.

('97). Neumann, Rudolf. Studien über die Variabilität der Farbstoffbildung bei Mikrococcus pyogenes α aureus (Staphylococcus pyogenes aureus) und einigen anderen Spaltpilzen. Arch. f. Hyg., Bd. XXX, 1897, pp 1-31. 1 table.

"Die eine Race kann also aus der anderen entstehen und in eine andere ubergeführt werden."

('98). Ward, H. Marshall. A violet bacillus from the River Thames. Annals of Botany, vol XII, 1898, pp. 59-74. One double plate in color.

('98). Niederkorn. See XV.

('98). Ruzicka. See XV.

('99). Jordan, Edwin O. The production of fluorescent pigment by bacteria. Botanical Gazette, vol. XXVII, pp. 19-36, 1899.

('99). Jordan, E. O. Bacillus pyocyaneus and its pigments. Jour. Exper. Med., vol. IV, Nos 5 and 6, 1899. pp. 627-647.

('99). Boland, G. W. Ueber Pyocyanin den blauen Farbstoff des Bacillus pyocyaneus. Centralb. f. Bakt., XXV Bd., 1899, pp. 897-902, with 1 curve.

Very probably B. pyocyaneus forms two pigments.

('00). Thiry, Georges. Bacille polychrome et Actinomyces mordoré. Recherches biologiques sur les bactéries bleues et violettes. Polychromisme. Corps bactériens et cristaux colorés. Matière colorante cristallisée. Travaux du lab. d'hyg. et de l'inst. sérothérapique de l'Univer. de Nancy. Paris, J. B Ballière et fils, 1900, pp. VIII, 154, 7 plates.

Contains also a bibliography of 141 titles on pigment-forming bacteria.

('00). Kuntze, W Ein Beitrag zur Kenntnis der Bedingungen der Farbstoffbildung des Bacillus prodigiosus. Zeitschr. f. Hyg., Bd. XXXIV, Hft. 1, 1900, pp. 169-184. Rev in Centralb f. Bakt., XXVIII Bd., 1900, pp 602-604

With a solution made up of 100 parts pure water, 1 to 2 asparagin, 2 to 4 c. p grape sugar, and 0.2 dipotassium phosphate, the author obtained a fairly good growth of B prodigiosus without color. With the same solution and a grape sugar not quite pure, there was always a formation of pigment. The white bacteria became pigmented in a few hours on potato, or on adding a trace of (0.001) of $MgSO_4$. This substance contaminated the sugar first used.

('00). Krause. See xv.

('00). Chamot, E. M., and Thiry, G. Studies on chromogenic bacteria. 1. Notes on the pigment of Bacillus polychromogenes. Bot Gaz., vol. xxx, 1900, pp. 378-393. 16 figs Also a separate.

('01). Gessard, C. Variété mélanogène du bacille pyocyanique Ann. de l'Inst. Pasteur, T. xv, 1901, pp. 817-831.

('02). Gessard, C. Essai sur la biologie du bacille pyocyanique. Ann. Inst. Pasteur, Paris, T. xvi, 1902, pp 313-330.

('02) Petrow, N. Über einen neuen roten Farbstoff-bildenden Bacillus. Arb. a. d. Bact. Institut der techn. Hochschule zu Karlsruhe, ii Bd., 3 Heft, 1902, pp. 271-291, with 1 plate.

Describes Bacillus subkiliensis.

('02). Loew, O., and Kozai, Y. Ueber Ernährungsverhältnisse beim Bacillus prodigiosus. Bull. of the College of Agric., Tokyo Imperial Univ, vol. v, 1902, No. 2, pp. 137-141. Also a separate.

A favorable medium for production of pigment and bacteriolytic enzyme is composed of peptone 1 per cent, sodium acetate 0.2 per cent, and asparagin 0.2 per cent in water.

('03). Marsh. See vi.

('03). Papenhausen, Hubert. Über die Bedingungen der Farbstoffbildung bei den Bakterien. Arb. a. d. Bact. Inst. der tech. Hochschule zu Karlsruhe, iii Bd., 1 Heft, 1903, pp. 43-79. Bibliog of 20 titles.

Twenty-two species experimented upon. Oxygen is very necessary for the production of the pigments. The other conditions for optimum production of pigment vary greatly in different species.

('04). Hefferan, Mary. A comparative and experimental study of bacilli producing red pigment. A dissertation submitted to the faculties of the Graduate Schools of Arts, Literature and Science, in candidacy for the degree of Doctor of Philosophy. The University of Chicago. Printed in Jena by Gustav Fischer, 1904, pp. 55 Bibliog. of 77 titles.

('04). Leonard, Ethel L. Bacterium cyaneum: A new chromogenic organism. The Johns Hopkins Hospital Bulletin, vol xv, 1904, pp. 398-400.

XXIV. Reduction and Oxidation.

('87). Spina, A. Bacteriologische Versuche mit gefarbten Nahrsubstanzen. Centralb. f. Bakt., ii Bd, Jena, 1887, Nos. 2-3, pp. 71-75

Reduction processes of bacteria in presence of methylen blue, etc.

('87). Cahen, Fritz. Ueber das Reduktionsvermogen der Bakterien. Zeitschr. f. Hyg., Bd. ii, 1887, pp. 386-396.

('91). Winogradsky. See xxv.

('94). Fermi, Claudio, and Montesano, Giuseppe. Ueber die Dekomposition des Amygdalins durch Mikroorganismen. Centralb. f. Bakt, Bd xv, 1894, pp. 722-727.

('96). Smith, Theobald. Reduktionserscheinungen bei Bakterien und ihre Beziehungen zur Bakterienzelle, nebst Bemerkungen über Reduktionserscheinungen in steriler Bouillon. Centralb. f. Bakt., xix Bd, 1896, pp. 181-187.

('99). Mueller, Friedr. Ueber reduzierende Eigenschaften von Bakterien. Centralb. f. Bakt., xxvi Bd., 1899, pp. 51-63.

The pigment used must be soluble in water, and must not poison the bacteria. Author uses methylene blue and litmus. Under literature, 11 papers are cited.

('99). Rothberger. See xviii.

('99) Mueller, Friedrich. Ueber das Reduktionsvermögen der Bakterien. Centralb. f. Bakt., xxvi Bd, 1899, pp 801-819.

Author used methylene blue, litmus, indigo-carmin and rosanilin acetate in various media with many organisms.

('01). Gran. See xxv.

('02). Cathcart, Eduard, und Hahn, Martin. Ueber die reduzierenden Wirkungen der Bakterien. Arch. Hyg., Munchen, Bd. xliv, 1902, pp. 295-321.

('02). Emmerling, Oscar. Die Zersetzung stickstofffreier organischer Substanzen durch Bakterien. Braunschweig (F. Vieweg & S.), 1902, pp. ix, 141, mit 7 Taf.

Not seen.

('03). van Delden, A Beitrag zur Kenntniss der Sulfatreduction durch Bacterien. Centralb. f. Bakt, 2 Abt., xi Bd., 1903, pp. 81-94 and 113-119, 1 heliotype pl.

XXV. Nitrifying and Denitrifying Organisms, Use of Free Nitrogen.

('66). Woronin. Ueber die bei der Schwarzerle und der gewöhnlichen Lupine auftretenden Wurzelanschwellungen. Mémoires de l'Acad. imp. St. Pétersb., 7 Serie, T. x, 1866. See also Ann. des sci. nat. Bot., 5 sé., T. vii, pp. 73-86, 1 plate

('77). Schloesing, Th., et Muentz, A. Sur la nitrification par les ferments organisés. C. R. des sé. de l'Acad. des sci., Paris, 1877, T. lxxxiv, p. 301; and T. lxxxv, p. 1,018.

('79) Schloesing, Th., et Muentz, A. Recherches sur la nitrification. C. R. des sé. de l'Acad. des sci, Paris, T. lxxxix, 1879, pp. 891-894 and 1,074-1,077.

('82). Déhéran et Maquenne. Sur la réduction des nitrates dans la terre arable. C. R. des sé. de l'Acad. des sci., 1882, T. xcv, pp. 691-693, 732-734, 854-856.

('82). Dupetit, G., et Gayon, U. Sur la fermentation des nitrates. C. R. des sé. de l'Acad. des sci., T. xcv, pp. 644-646, Paris, 1882.

('82). Dupetit, G., et Gayon, U. Sur la transformation des nitrates en nitrites. C. R. des sé. de l'Acad des sci, T. xcv, Paris, 1882, pp. 1,365-1,367.

('83). Dupetit, G., et Gayon, U. Quelques-unes des conditions les plus favorables à la fermentation des nitrates Mém de la soc. des sci. physiques et naturelles de Bordeaux, 2 sé., T. v, 1883, Extr. Procès-verbaux sé. du 23 nov., 1882, pp. iii-iv.

('83). Springer, A. Reduction of nitrates by ferments. Am. Chem. Jour., vol. iv, pp. 452-453, 1883.

('86). Gayon et Dupetit. Recherches sur la réduction des nitrates par les infiniments petits., 1886. Paris,? Publisher?

Not seen.

('86). Munro, J. H. M. The formation and destruction of nitrates and nitrites in artificial solutions, and in river and well waters. Jour. Chem. Soc. Trans., London, 1886, vol. xlix, pp. 632-681.

('88). Beyerinck. Die Bakterien der Papilionaceenknöllchen. Bot. Zeitung, Jahrg. xlvi, 1888, col. 725-735, 741-750, 757-771, 781-790, 797-804, 2 plates. Review in Centralb. f. Bakt., Bd. v, 1889, pp. 804-805.

('88). Hellriegel, H., und Wilfarth, H. Untersuchungen uber die Stickstoffnahrung der Gramineen und Leguminosen, 1888. Beilageheft zu der Zeitschr. d. Vereins f. d. Rübenzucker-Industrie d. Deutschen Reichs. Berlin, pp. 234, with 6 plates.

('90). Frankland, Percy F. The nitrifying process and its specific ferment, Pt. i. Phil. Trans Roy. Soc., Lond., vol. 181 B, 1890, pp. 107-128.

('90). Winogradsky, S. Recherches sur les organismes de la nitrification Ann. de l'Inst. Pasteur, T. iv, 1890, pp. 213-231, 257-275, and 760-771.

Nitromonas can live on purely inorganic matters, although destitute of chlorophyll His "nitratferment" consists of pear-shaped rods, which do not exceed ½ μ in length, and are 1½ to 2 times less in breadth. The "nitritorganismus" is 4 to 5 times larger than the "nitrathildner," and has a roundish form.

('90). Laurent, Em. Expériences sur la réduction des nitrates par les végétaux. Ann. de l'Inst. Pasteur, T. iv, pp. 722-744, 1890.

('91). Giltay, E, und Aberson, J H. Recherches sur un mode de dénitrification et sur le schizomycète qui la produit. Archives néerlandaises des sci. ex. et nat., 1891, T. xxv, pp. 341-361, 1 plate.

('91). Schloesing, Th., fils, et Laurent, Emile. Sur la fixation de l'azote libre par les plantes. C. R. des sé. de l'Acad. des sci. Paris, 1891, T. cxiii, pp. 776-778 and 1,059-1,060, 1892, T. cxv, pp. 659-661 and 732-735.

('91). Winogradsky, S. Recherches sur les organismes de la nitrification. Ann. de l'Inst. Pasteur, T. v, 1891, pp. 92-100 and 577-616.

Winogradsky's fourth and fifth memoirs on nitrifying bacteria. The fourth deals with the question of solid media suited to their culture, and describes the making of silicate jelly. The fifth deals with the formation and the oxidation of nitrites in the nitrification.

('91). Beijerinck, M. W. Over ophooping van atmospherische stikstof in culturen van Bacillus radicicola. Versl. en Mededeel. der Koninkl. Akad. van Wetensch. zu Amsterdam, Afd. Natuurkunde, iii, 8, 1891, pp. (460)-(475), 1 text fig. Abstract in Koch's Jahresb, iii Bd., p. 205.

('92). Winogradsky, S. Contributions à la morphologie des organismes de la nitrification. Arch. d sci. biol. publiées par l'Institut Impérial de méd. expér. à St. Pétersbourg, Tome i, 1892, p. 108 Reviewed in Ann. de l'Inst. Pasteur, T. vi, 1892, pp 458-462.

('92). Schloesing, Th., fils, et Laurent, Emile. Recherches sur la fixation de l'azote libre par les plantes Ann. de l'Inst. Pasteur, 1892, T. vi, pp. 65-115.

('92). Berthelot. Nouvelle recherches sur la fixation de l'azote par les microbes. C. R. des sé de l'Acad. des sci, 1892, T cxv, pp. 569-574

('93). Berthelot. Recherches nouvelles sur les microorganismes fixateurs de l'azote. C. R. des sé. de l'Acad. des sci., Paris, 1893, T. cxvi, pp. 842-849

('94). Duclaux, E. Sur la fixation de l'azote atmosphérique. Revue critique. Ann. de l'Inst. Pasteur, 1894, T. viii, pp. 728-736.

('94). Winogradsky, S. Sur l'assimilation de l'azote gazeux de l'atmosphere par les microbes C. R. des sé. de l'Acad. des sci., Paris, T. cxvi, 1893, p. 1,385, and T. cxviii, 1894, Feb. 12, p. 353. Rev. in Centralb. f. Bakt., Bd xvi, 1894, pp. 129-130.

('95). Winogradsky, S. Recherches sur l'assimilation de l'azote libre de l'atmosphère par les microbes. Arch. des sci. biol. pub. par l'Institut. imperial de médecine expérimentale à St. Pétersbourg, Tome iii, No. 4, 1895, pp. 297-352.

A very interesting and valuable paper.

('96). Winogradsky, S. Zur Mikrobiologie des nitrifikationsprozesses. Centralb. f. Bakt., 2 Abt., Bd. ii, 1896, pp. 415-428.

('97). Mazé. Fixation de l'azote libre par le bacille des nodosités des légumineuses. Ann. de l'Inst Pasteur, T. xi, 1897, pp. 44-54.

The second and third memoirs are continued in the Annales for 1898, under the title, "Les microbes des nodosités des légumineuses."

('98). Konvalewski, S. Beiträge zur Frage über die Assimilierung von freiem Stickstoff seitens der Bakterien. Russ. Arch. f. Pathol., Bd. vi, 1898, p. 251. Rev. in Centralb. f. Bakt., xxv Bd., 1899, pp. 771-772.

('98). Mazé. Les microbes des nodosités des légumineuses. Ann. de l'Inst. Pasteur, T. xii, 1898. Second memoir, pp. 1-25, 1 fig. Third memoir, pp. 128-155, 2 plates.

The second memoir deals with the physiology of the organism. The third memoir deals with the morphology of the root-tubercle organism. Mazé says it is pathogenic for certain animals. In the animal body it is said to become nearly round, to be much reduced in diameter, and to have a tendency to grow in chains.

('99). Oméliansky, V. Sur la nitrification de l'azote organique. Arch. des sci. biol. publiées par l'inst imp. de méd. expér. à St. Pétersbourg, T. vii, 1899, pp. 272-290.

('99). Oméliansky, V. Sur la culture des microbes nitrificateurs du sol. Arch. des sci. biol. publiées par l'inst. imp. de méd. expér. à St. Pétersbourg, T. vii, 1899, pp. 291-302

(99). Winogradsky u. Oméliansky. Ueber den Einflus der organischen Substanzen auf die Arbeit der nitrificierenden Mikrobien. Centralb. f. Bakt, 2 Abt., Bd. vi, 1899, p. 329

('99). Winogradsky, S., et Oméliansky, V. L'influence des substances organiques sur le travail des microbes nitrificateurs. Arch. des sci. biol. publiées par l'inst. imp. de méd. expér. à St. Pétersbourg, T. vii, 1899, pp. 233-271.

('00). Krueger, W., and Schneidewind, W. Ursache und Bedeutung der Salpeter-Zersetzung im Boden. Landwirtschaftliche Jahrbücher. Ztschr. f. wiss. Landwirtschaft. Berlin, 1900, Bd xxix, pp. 747-770, 2 plates.

('01). Beijerinck, M. W. Ueber oligonitrophile Mikroben. Centralb. f. Bakt., etc., Bd. vii, 1901, pp 561-582, with 1 heliotype plate.

Describes Azotobacter

('01). Gran, H. H. Studien über Meeresbakterien. I. Reduction von Nitraten und Nitriten. Bergens Museums Aarbog., 1901, No. 10, pp. 23.

Three new species are described: Bacillus repens, B. trivialis, B Hensenii.

('01). Maassen, Albert. Die Zerzetzung der Nitrate und der Nitrite durch die Bakterien. Ein Beitrag zum Kreislauf des Stickstoffs in der Natur. Arbeit. a. d. K. Gesundheitsamt., Bd. XVIII, 1901, pp. 21-77.

('02). Moore, Geo. T. Bacteria and the Nitrogen Problem. Yearbook Dept. Agric., 1902, pp. 333-342, 6 plates. Also a separate.

Pure cultures of the root-tubercle organism are absorbed on cotton, which is then dried, wrapped in tin foil, and thus distributed to the agriculturist. He is directed to throw the cotton into a pail of water, to which is added small packages of nutrient salts. After 48 hours the fluid is filled with the bacterial growth. The leguminous seeds are then drenched with it, and sown after drying in the shade.

('02). Smith, R. Greig. Notes on Vibrio denitrificans, Sewerin. Proc. Linnean Soc., New South Wales, for the year 1901. Sydney, 1902, vol. XXVI, Pt. I, pp. 118-121. I plate.

('02) Omélianski, W. Kleinere Mitteilungen über Nitrifikationsmikroben. I. Die Kultur des Nitritbildners auf Papierscheiben. II. Wird schweflige und phosphorige Säure durch Nitrobacter oxydiert? III. Scheiden die Nitritmikroben eine Oxydase aus? Centralb. Bakt., Abt 2, Bd. VIII, 1902, pp. 785-787; Bd. IX, 1902, pp. 63-65, pp. 113-117, mit I Taf.

('02). Beijerinck, M. W., und van Delden, A. Ueber die Assimilation des freien Stickstoffs durch Bacterien. Centralb Bakt., Abt. 2, Bd. IX, 1902, pp. 3-43.

('02). Buhlert, H. Untersuchungen über die Arteinheit der Knöllchenbakterien der Leguminosen und über die landwirtschaftliche Bedeutung dieser Frage. Habilitationsschr. Halle (Druck v. Wischan u. Wettengel), 1902, p. 55 Centralb. Bakt., Abt. 2, Bd. IX, 1902, pp. 148-153, 226-240, and 273-285.

('02) Hiltner, L. Ueber die Impfung der Leguminosen mit Reinkulturen. D. landw. Presse, Berlin, Bd. XXIX, 1902, pp. 119-120.

('02). Nobbe, Friedrich, und Richter, L. Ueber den Einfluss des Nitratstickstoffs und der Humussubstanzen auf den Impfungserfolg bei Leguminosen. Landw. Versuchstat., Berlin, Bd. LVI, 1902, pp. 441-448.

('03). Bersteyn, P. Über einige in den Kulturen zur Reinzüchtung der Nitratbildner regelmässig auftretende Bakterienarten. Arb. a. d. Bact. Inst. der tech. Hochschule zu Karlsruhe, III Bd., I Heft, 1903, pp. 81-100.

The following new species are described: Bacterium comes, B. modestum, B. debile and Ps humicola. None of these species will grow in media *entirely free* from organic matter.

('03). Benecke, W., und Keutner, J. Ueber stickstoffbindende Bakterien aus der Ostsee. Ber. d. deutsch. bot. Gesellsch., Bd. XXI, Heft 6, pp. 333-346, Berlin, 1903, 4 text figs.

('05). Moore, George T. Soil Inoculation for Legumes. Bureau of Plant Industry, U. S. Department of Agriculture, Bull. 71, Jan. 23, 1905, pp. 72, pl. 9.

XXVI. Use of Free Carbon Dioxide.

('87). Hueppe, Ferdinand. Ueber Chlorophyllwirkung chlorophyllfreier Pflanzen. Tageblatt der 60 versammlung deutscher Naturforscher u. Aerzte in Wiesbaden, 1887, pp. 244-245.

('90). Winogradsky u. Omélianski. See XXV.

('02) Nathansohn. See LI.

('03). Beijerinck, M. W., und van Delden, A. Ueber eine farblose Bakterie, deren Kohlenstoffnahrung aus der atmosphärischen Luft herrührt. Centralb. f Bakt., 2 Abt., Bd. X, No. 2, 1903, pp. 33-47. See also Versl. Wis. Nat. Afd K. Acad. Wet., Bd. XI, 1903, pp. 450-465 (Dutch); and Proc. Sci. K. Acad. Wet., Bd. V, 1903, pp. 398-413 (English), Amsterdam.

"We will use the name Bacillus oligocarbophilus to designate a colorless bacterium whose carbon needs, in the dark as well as in the light, are satisfied by a not yet exactly known carbon compound, or compounds, of the air, out of which compound this organism must also create the necessary energy for its life processes." It is stated that this substance is not carbon dioxide.

('04). Beijerinck, M. W. Ueber die Bakterien, welche sich im Dunkeln mit Kohlensäure als Kohlenstoffquelle ernähren können. Centralb. f. Bakt., 2 Abt., XI Bd., 1904, No. 20-22, pp 593-599.

XXVII. Luminous Bacteria.

('75). Pflueger, E. Beiträge zur Lehre von der Respiration. Sec. 5. Die Phosphorescenz der lebendigen Organismen und ihre Bedeutung für die Principien der Respiration. Pflüger's Archiv, 1875, Bd. X, pp. 275-300.

('80). Lassar, O. Die Micrococcen der Phosphorescenz. Pfluger's Arch. f. die gesam. Physiol., 1880, Bd. XXI, pp. 104-108.

('85). Nueech. Ueber leuchtende Bacterien. Bâle, 1885.

Not seen.

('87) Fischer. Bakteriologische Untersuchungen auf einer Reise nach Westindien. II. Ueber einen lichtentwickelnden, im Meerwasser gefundenen Spaltpilz. Zeitschr. f. Hyg. Bd. II, 1887, pp. 54-92, and Nachtrag, pp. 92-95.

('87). Forster, J. Ueber einige Eigenschaften leuchtender Bakterien. Centralb. f. Bakt., Bd. II, 1887, pp. 337-340.

('87). Katz, Oscar. Preliminary remarks on phosphorescent bacteria from sea-water. Proc. Linn. Soc., New South Wales, vol. II, Pt. II, 1887, pp 331-336.

('87). Duclaux. Sur les microbes phosphorescents. Revue critique. Ann. de l'Inst. Pasteur, T. I, 1887, pp. 489-492.

('88). Dubois, R. Sur le rôle de la symbiose chez certains animaux marins lumineux. C. R. des sé. de l'Acad. des sci., T. CVII, Paris, 1888, pp. 502-504

('88). Fischer, B. Ueber einen neuen lichtentwickelnden Bacillus. Centralb. f. Bakt., 1888, III Bd., pp. 105-108, and pp. 137-141.

('89). Beyerinck, M. W. Le photobacterium luminosum, bactérie lumineuse de la mer du nord. Arch. néer. des sci. ex. et nat., T. XXIII, 1889, pp. 401-415.

('89). Beyerinck, M. W. Les bactéries lumineuses dans leurs raports avec l'oxygène. Arch. néer. des sci. ex. et nat., T. xxiii, 1889, pp. 416-427.

('89). Giard, A., und Billet, A. Observations sur la maladie phosphorescente des Talitres et autres Crustacés. C. R. de la Soc. de biol., 1889, Tome i, pp. 593-597.

('89). Lehmann, K. B. Studien über Bacterium phosphorescens Fischer. Centralb. f. Bakt., v Bd., 1889, pp. 785-791.

('90). Giard, A. Nouvelles recherches sur les bactéries lumineuses pathogènes. C. R. de la Soc. de biol., 1890, Tome ii, pp. 188-191.

('90). Beijerinck, M. W. Over lichtvoedsel en plastisch voedsel van lichtbacteriën. Versl. en Meded. d. Kon. Akad. v. Wetenschappen, Aft. Natuurk. Derde Reeks, Deel vii, 1890, pp. 239-302. 1 text figure.

('90). Billet, A. Contribution à l'étude de la morphologie et du développement des bactériacées. Bull. scientifique de la France et de la Belgique, publié par Giard, T. xxi, 1890. pp. 1-289, plates 9. Bibliography of 662 titles. Also a separate.

The bacterium of luminous sand fleas is described in a note on p. 144 as Bacterium Giardi Billet.

('91). Beyerinck, M. W. Sur l'aliment photogène et l'aliment plastique des bactéries lumineuses. Arch. néerl. des sci. ex. et nat., T. xxiv, 1891, pp. 369-442.

('91). Katz, Oscar. Zur Kenntniss der Leuchtbakterien. Centralb. f. Bakt., 1891, ix Bd., pp. 157-163, 199-204, 229-234, 258-264, 311-316, 343-349.

('92). Russell, H. L. Impfungsversuche mit Giard's pathogenem Leuchtbacillus. Centralb. f. Bakt., xi Bd., 1892, pp. 557-559.

Author obtained no conclusive evidence as to the pathogenic nature of this organism.

('92). Eijkmann, C. Lichtgevende Bacteriën Geneeskundig Tijdschrift voor Nederlandsch-Indië. Deel xxxii, Aflevering 4, Batavia en Noordwijk, 1892, pp. 109-115. Also a separate. Rev. in Centralb. f. Bakt., xii Bd., 1892. pp. 656-657.

('93). Dubois, Raphael. Extinction de la luminosité du Photobacterium sarcophilum par la lumière. C. R. des sé. et mém. de la soc. de Biologie, Paris, 1893, 9 sér., T. v, pp. 160-161.

This organism was isolated from the luminous flesh of a dead rabbit. It was cultivable in a synthetic medium made as follows: Ordinary water, 100; asparagine, 1; glycerin, 1; potassium phosphate, 0.10; sea salt, 3. After some months in this medium the luminosity diminished. Exposed to the light for some days, at about 10° C., the culture takes on a fine orange yellow color, becomes opaque, and loses its luminosity except at the edges. Transfers may be made from this yellow growth readily, but the resulting cultures are not luminous. In the dark, however, such cultures return after some days to their orginal transparent color, and again become luminous.

('95). Kutscher. Zur Phosphorescens der Elbvibrionen. Centralb f. Bakt., xviii Bd., 1895, p 424.

('98) Frankland, Percy. The action of bacteria on the photographic plate Centralb. f Bakt, xxiv Bd., 1898, pp 600-612.

Only the luminous sorts are able to act through glass. The action of non luminous species is probably due "to the evolution of volatile chemical substances" The latter exposures were long—several days

('99). Henneberg, W. Leuchtbakterien als Krankheitserreger bei Schwammücken. Centralb. f. Bakt., xxv Bd., 1899, pp. 649-650.

('99). Barnard, J. E. Photogenic Bacteria. Trans. of the Jenner Inst., London, 1899, second series, pp. 81-112. 2 plates.

"Spectroscopically the light emitted by the photogenic organisms examined by me is confined to a very small portion of the visible spectrum; never extending into the ultra-violet or infra-red. Visually it only includes the green and blue, and photographically it extends very slightly further into the violet. The economic value of such a light source is obvious, and it is a matter of great practical importance to determine its method of production."

The writer experimented with 13 species.

('02). McKenney, R. E. B. Observations on the conditions of light production in luminous bacteria. Proc. of Biol. Soc. of Wash., Nov. 20, 1902, vol. xv, pp. 213-234. Also a separate. Bibliog. of 35 titles.

('02). Barnard, J. E., and Macfadyen, Allan. On Luminous Bacteria. Annals of Botany, vol. xvi, Dec., 1902, pp. 587-588.

('03). Molisch, Hans. Vienna Acad. Sci., 1903. Luminous bacteria for Safety Lamp. Science, 1903, p. 719.

Paper read, but not yet printed. Note in Science made from a Reuter's telegram.

('04). Gorham, F. P.

See a preliminary note in Centralb. f. Bakt., 2 Abt., xiii Bd.

XXVIII. Hydrogen Sulphide and Otherwise Unclassified By-Products.

('79). Miquel, P. De la fermentation sulfhydrique Bull. de la Soc. chim., T. xxxii, 1879, p. 127-131.

('89). Holschewnikoff. Ueber die Bildung von Schwefelwasserstoff durch Bakterien. Fortschr. d. Med., 1889, Bd. vii, pp. 201-213

('91). Nencki, M. Die isomeren Milchsäuren als Erkennungsmittel einzelner Spaltpilzarten. Centralb. f. Bakt., Bd. ix, 1891, pp. 304-306.

('91-'93). Ray-Pailhade. Recherches expérimentales sur le philothion. Paris, 1891. Le philothion et le soufre. Assoc. franç. pour l'av. des sci., Congrès de Besançon, 1893. Part i, pp. 193, 250, and 302.

('92). Sommaruga, E. Ueber Stoffwechselproducte von Mikroorganismen. Zeitschr. f. Hyg., Bd. xii, 1892, pp. 273-297.

('92). Petri, R. J., u. Maassen, A. Ueber die Bildung von Schwefelwasserstoff durch die Krankheitserregenden Bakterien unter besonderer Berücksichtigung des Schweinerothlaufs. Veröffent. d. k. Gesundheitsamtes, xvi Jahrg., 1892, p. 119.

('93). Stagnitta-Balistreri. Die Verbreitung der Schwefelwasserstoffbildung unter den Bakterien. Arch. für Hyg., Bd. xvi, 1893, pp. 10-34.

For demonstration of sulphuretted hydrogen in gelatin, the author adds iron saccharate, tartrate, or acetate to the gelatin. For fluids he uses lead acetate paper.

('93). Rubner. Ueber den Modus der Schwefelwasserstoffbildung bei Bakterien. Arch. f. Hyg., Bd xvi, 1893, pp. 53-72.

('93). Rubner. Die Wanderungen des Schwefels im Stoffwechsel der Bakterien. Arch. f Hyg., 1893, Bd xvi, pp 78-100

('93). Petri, R. J., und Maassen, Albert. Beiträge zur Biologie der krankheitserregenden Bakterien ins besondere uber die Bildung von Schwefelwasserstoff durch dieselben unter vornehmlicher Berücksichtigung des Schweinerothlaufs. Arbeiten aus dem Kaiserl Gesundheitsamte, Bd. VIII, 1893, pp. 318-356.

Many parasitic and saprophytic forms have been tested. All produce sulphuretted hydrogen when cultivated in presence of suitable sulphur compounds, i. e., such as are loosely bound. The reduction of litmus and indigo in solid cultures is ascribed to this gas. It is believed that all bacteria are capable of producing it, and that the distinction into producers and non-producers of sulphuretted hydrogen must be abandoned.

('93). Petri, R. J., und Maassen, Albert. Weitere Beiträge zur Schwefelwasserstoffbildung aërober Bakterien und kurze Angaben über Merkaptanbildung derselben. Arbeiten aus dem Kaiserlichen Gesundheitsamte, Bd. VIII, 1893, pp. 490-506.

('93). Rubner, M. Ueber das Vorkommen von Mercaptan. Archiv. f. Hyg., 1893, Bd. XIX, pp. 136-193.

('94). Wenzell, W. T. A contribution to the knowledge of bacteriologic chemistry. Jour. Am. Med. Asso., 1894, vol. XXIII, pp. 901-903.

('96). Duclaux, E. Sur les odeurs de putréfaction. Revue critique Ann. de l'Inst. Pasteur, T. X, 1896, pp. 59 to 64.

('97). Morris, Max. Studien über die Produktion von Schwefelwasserstoff, Indol und Merkaptan bei Bakterien. Arch. f. Hyg., XXX Bd., 1897, pp. 304-311.

Mercaptan was demonstrated by use of "isatinschwefelsaure," which is colored green by this substance.

('02). Banning, Friedrich. Zur Kenntniss der Oxalsäurebildung durch Bakterien. Centralb. f. Bakt., Abt. 2, Bd. VIII, 1902, pp. 520-525, pp. 556-567, mit 1 Taf.

XXIX. Action of Light on Bacteria.

('77). Downes and Blunt. Researches on the effect of light upon bacteria and other organisms. Proc. Roy. Soc., vol. XXVI, No. 184, 1877, 6 Dec., pp. 488-500. London.

Downes and Blunt determined germicidal action to be associated chiefly with the actinic rays of the spectrum.

('78). Downes and Blunt. On the influence of light upon protoplasm. Proc. Roy. Soc., vol. XXVIII, No. 191, 1878, 19 Dec., pp. 199-212.

('78). Tyndall, J. Note on the influence exercised by light on organic infusions. Proc. Roy. Soc., London, vol. XXVIII, pp. 212-217. Nature, vol. XIX, 1879, p. 210.

Negative results. Used flasks, and organisms grew after exposure to sunlight.

('81). Tyndall, John. On the arrestation of infusorial life by solar light. Br. asso. for the advancement of sci. Rep., 1881, pp. 450-451. Nature, vol. XXIV, p. 466, 1881.

Tyndall's results were due to the way in which he experimented, i. e., with *flask* cultures.

('82). Engelmann, Th. W. Bacterium photometricum. Ein Beitrag zur vergleichenden physiologie des Licht- und Farbensinnes. Onderzoekingen gedaan in het Physiologisch Laboratorium der Utrechtsche Hoogeschool, Derde Reeks, VII, Aflev. II, 1882, pp. 252-290, 1 plate.

('84). Downes, A., and Blunt, T. P. The influence of light on bacteria. Trans. Roy. Soc., Victoria, vol. XX, pp. 1-2, 1884.

Reply to Jamieson.

('85). Duclaux, E. Influence de la lumière du soleil sur la vitalité des germes de microbes. C. R. des sé. de l'Acad. des sci. T. C., 1885, pp. 119-121.

('85). Duclaux, E. Influence de la lumière du soleil sur la vitalité des micrococcus. C. R. des sé. de l'Acad. des sci., Paris, T. CI, 1885, pp. 395-397.

('86). Arloing, S. Influence de la lumière blanche et de ses rayons constituants sur le dévéloppement et les propriétés du Bacillus anthracis. Arch. de physiol. normale et pathol., 1886, 3 sér., T. VII, pp. 209-235.

('86). Straus, I. Note sur l'action de la lumière solaire sur les spores du Bacillus anthracis. C. R. des sé. et mém. de la soc. de Biologie. Paris, 8 sé., T. III, 1886, pp. 473-474.

('86). Downes, Arthur. On the action of sunlight on Micro-organisms with a demonstration of the influence of diffused light.—Proceedings of the R. Society, London, 1886, vol. XL, pp. 14-22.

('87). Duclaux, E. Action de la lumière sur les microbes. Ann. de l'Inst. Pasteur, 1887, T. I, pp. 88-92.

('87). Roux, E. De l'action de la lumière et de l'air sur les spores de la bactéridie du charbon. Ann. de l'Inst. Pasteur, T. I, 1887, pp. 445-452.

('88). Engelmann, Th. W. Die Purpurbakterien und ihre Beziehungen zum Lichte. Bot. Zeitung, 1888, Jahrgang XLVI, col. 661, 677, 693, and 709.

('88). Engelmann, Th. W. Ueber Bacteriopurpurin und seine physiologische Bedeutung. Pfluger's Archiv., 1888, Bd. XLII, pp. 183-186.

('89). Raum, Johannes. Der gegenwartige Stand unserer Kenntnisse über den Einfluss des Lichtes auf Bacterien und auf den thierischen Organismus. Zeit. f. Hyg., Bd. VI, 1889, pp. 312-368.

This paper contains a bibliography of 7 pages.

('89). Pansini, S. Dell'azione della luce solare sui microorganismi. Revista d'igiene practica e sperimentale, Napoli, 1889, pp. 69-101. Review in Ann. de Micr., 1890, p. 516.

('90). Saverio. L'influenza della temperatura sull' azione microbicida della luce. Ann. dell' Inst. d'ig. di Roma.

Not seen.

('90). Janowski, Th. Zur Biologie der Typhusbacillen. Centralb. f. Bakt., VIII Bd., 1890, pp. 167-172, 193-199, 230-234, and 262-266.

Discusses the action of sunlight.

('91). Geisler, F. K. On the action of light on bacteria. (Russian.) Wratsch, 1891, No. 36, pp. 793-797.

Not seen.

('92). Momont. See XXXV.

('92). Kotljar, E. Zur Frage über den Einfluss des Lichtes auf Bakterien. Wratsch, 1892, Nos. 39 and 40. Rev. in Centralb. f. Bakt., XII Bd., 1892, p. 836. Also in Ann. de l'Inst. Pasteur, T. VII, 1893, p. 430.

('92). Chmelewsky. Zur Frage über die Wirkung des Sonnen- und elektrischen Lichtes auf die Eiterbakterien. Wratsch, 1892, No. 20. Reviewed in Centralb. f. Bakt., Bd. xii, pp. 174-175, 1892.

('92). Geisler, Theodor. Zur Frage über die Wirkung des Lichtes auf Bakterien. Centralb. f. Bakt., xi Bd., 1892, pp. 161-173.

('92). Buchner, H. Ueber den Einfluss des Lichtes auf Bakterien. Centralb. f. Bakt., xi Bd., 1892, pp. 781-783.

Deals with question of effect of light on bacteria suspended in water "The result of all these experiments points to the conclusion that light exerts a powerful disinfecting influence upon the named bacterial sorts when these are suspended in water." The experiments were made on typhoid bacilli, B. coli communis, B. pyocyaneus, cholera vibrios, and various bacteria of decay.

('92). Buchner, H. Ueber den Einfluss des Lichtes auf Bakterien. ii Mitth. Centralb. f. Bakt., Bd. xii, 1892, Heft 7-8, pp. 217-219, 1 fig.

Author obtained bacterial letters and figures on agar and gelatin plates by covering a part and exposing to sunshine. Light passed through water still possesses active bactericidal powers.

('93). Buchner, H. Ueber den Einfluss des Lichtes auf Bakterien und über die Selbstreinigung der Flüsse. Arch. f. Hyg., Bd. xvii, 1893, pp. 179-204.

('93). Richardson, Arthur. The action of light in preventing putrefactive decomposition; and in the formation of hydrogen peroxide in organic liquids. Jour. Chem. Soc., Transactions, London, 1893, vol. lxiii, pp. 1,109-1,130.

('94). d'Arcy, R. F., and Hardy, W. B. Note on the oxidizing powers of different regions of the spectrum in relation to the bactericidal action of light and air. Jour. of Physiol., 1894-'95, vol. xvii, pp. 390-393.

('94). Ward, H. Marshall. The Action of light on bacteria. Phil. Trans Royal Soc., Lond., vol. 185 (1894), pp. 961-986. Also a separate. 4to., 25 pp, 1 plate. See also Revue Sci., 1894.

('94). Fischer, Bernhard. Die Bakterien des Meeres nach den Untersuchungen der Planktonexpedition unter gleichzeitiger Berücksichtigung einiger älterer und neuerer Untersuchungen Centralb. f. Bakt, Bd. xv, 1894, pp 657-666

Shows that sunlight tends to reduce number of bacteria in upper layers of the sea There are always more bacteria in upper layers of the sea at sunrise than in the afternoon There are also in the daytime a great many more at a depth of 10 meters than near the surface. The depth to which the bactericidal action of the sunlight penetrates depends on its intensity, duration of action, clearness of the water, etc. It probably reaches down several meters. Cultures of various water bacteria were killed in a short time when exposed to the midday sun in August, the sun's rays being first passed through one-half meter of sea water.

('94). Dieudonné, A. Beiträge zur Beurteilung der Einwirkung des Lichtes auf Bakterien. Arbeiten a. d. Kaiserl. Gesundheitsamte, Bd. ix, 1894, Heft 2, pp 405-413. Rev. in Centralb. f Bakt, xvii Bd, 1895, pp 646-647.

The red end of the spectrum (between the lines D and E) has no injurious effect. In the green (between lines E and F) there is a distinct inhibiting action. In the blue-violet and ultra-violet the bactericidal action is most marked. The action of the light is directly on the bacteria. Bacteria inoculated in plates already exposed to the light grew just as well as in the control plates Light, which has had the heat rays removed by filtration through alum solution, possesses the same germicidal action.

('94). Dieudonné, A. Ueber die Bedeutung des Wasserstoffsuperoxyds für die bakterientödtende Kraft des Lichtes. Arbeiten aus dem kais. Gesundheits-Amt., Bd. ix, 1894, pp. 537-540.

Ascribes bactericidal effect of light in great part to the formation of hydrogen peroxide in the culture-medium.

('94). Engelmann. See xix.

('94). d'Arsonval and Charrin. See xxxiii.

('96). Beck, M., u. Schultz, P. Ueber die Einwirkung sogen. monochromatischen Lichtes auf die Bacterienentwicklung. Zeitschr. f. Hyg, Bd. xxiii, 1896, pp. 490-496.

('99). Kedzior, Laurenz. Ueber den Einfluss des Sonnenlichtes auf Bakterien. Arch. f. Hyg., Bd xxxvi, 1899, pp 323-334. Rev. in Centralb. f. Bakt., Bd. xxvii, 1900, pp. 203 and 759.

Sunlight also destroys in an atmosphere of hydrogen, although less actively.

('01). Simoncini, G. B., e Viola, D. L'influenze dell'innaffiamento sul contenuto batterico delle polveri di strada. Ann. d'igiene sper., Roma, vol. xi, 1901, pp. 373-392.

Bibliog. of 21 titles. The bactericidal action of light was greater on the moistened dust of the street than on the dry dust.

XXX. Effect of Electricity.

('91). Spilker, W., und Gottstein, A. Ueber die Vernichtung von Mikroorganismen durch die Induktionselektricität. Centralb. f. Bakt., ix Bd., 1891, pp 77-88.

('91). Fermi, Claudio. Ueber die Reinigung der Abwässer durch Elektricität. Arch. für Hyg., Bd. xiii, 1891, pp. 207-228.

('93). Krueger, S. Ueber den Einfluss des constanten elektrischen Stromes auf Wachsthum der Bakterien. Zeitschr. f. klin med., Bd. xxii, 1893, pp. 191-207

Krueger's conclusions are: The inhibition or destruction of the bacteria is due to the liberation of ions in the fluid.

('93). Burci, E., e Frascani, V. Contributo allo studio dell'azione battericida della corrente continua.—Atti della Soc. Tosc. di Scienze nat. Pisa. Mem., vol. xii, 1893, pp. 99-119.

('94). d'Arsonval and Charrin. See xxxiii.

('96). Friedenthal, H. Ueber den Einfluss des elektrischen Stromes auf Bakterien. Kritisches Referat. Centralb. f. Bakt., xix Bd., 1896, pp. 319-324.

('96). Gottstein, A. Ueber den Einfluss des elektrischen Stromes auf Bakterien. Centralb. f. Bakt., xix Bd., 1896, pp 602-605.

('96). Friedenthal, H. Ueber den Einfluss des Induktionselektrizität auf Bakterien. Kritisches Referat. Centralb. f. Bakt., xx Bd., 1896, pp. 505-508.

('96) Marmier, L. A. Les toxines et l'électricité Ann. de l'Inst. Pasteur, T. x, 1896, pp. 469-480.

('99). Thiele, Hermann, und Wolf, Kurt. Ueber die Einwirkung des elektrischen Stromes auf Bakterien Centralb. f Bakt., xxv Bd., 1899, pp. 650-655, with 1 fig.

Results all negative.

('00) Krause. See xv.

('01). Strebel, Hermann. Untersuchungen über die bakterieide Wirkung des Hochspannungsfunkenlichtes nebst Angabe einer Methode zur besseren Ausnützung der baktericiden Kraft des Voltabogenlichtes. D. med. Wochenschr., Berlin, Bd. xxvii, 1901, pp. 69-72, pp. 87-89.

('01). Ullmann, Johannes. Ueber die Einwirkung elektrischen Bogenlichts auf Mikroorganismen in Gegenwart von fluoreszierenden Stoffen. Diss. München (Druck v. M. Ernst), 1901, p. 17.

XXXI. Action on Bacteria of Roentgen Rays, Radium Rays, Etc.

('96). Wittlin, J. Les rayons Röntgen exercent-ils une action quelconque sur les bacteries? Ann. de micro., T. viii, 1896, pp. 514-515.

Author finds that the Roentgen rays have no effect upon bacteria.

('96). Minck, F. Zur Frage über die Einwirkung der Röntgen'schen Strahlen auf Bakterien und ihre eventuelle therapeutische Verwendbarkeit. Münchener mediz. Wochenschrift. 1896, Bd. xliii, pp. 101-102 and p. 202.

Author obtained only negative results.

('97). Pott, Francis. Concerning the action of X-rays on cultivation of tubercle Bacillus. The Lancet, London, vol. ii, for 1897 (55th year), pp. 1,314-1,315.

The tubercle bacillus was not affected by X-rays.

('97). Blaise et Sambuc. De l'action des rayons X sur le Pyocyaneus et la bactéridie charbonneuse. C. R. des sé. et mém. de la soc. de biol., T. iv, 10e série, 1897, pp. 689-692.

Little or no effect on these organisms.

('97). Beauregard et Guichard. Action des rayons X sur certains charactères biologique des microbes. C. R. des sé. et mém. de la soc. de biol., T. iv, 10e série, 1897, pp. 803-804.

The bacteria are much less sensitive than higher organisms.

('98). Rieder, Hermann. Wirkung der Röntgenstrahlen auf Bakterien. Münch. med. Wochenschr., 45 Jahrg., 1898, pp. 101-104, 2 text figures (exposed agar plates).

Contrary to the statements of various other experimenters, this writer says that he obtained positive germicidal results on seven pathogenic organisms by exposures lasting from 15 minutes to 1 hour. The earlier literature is cited. Voltohm's apparatus was used. The photographs show the center of the agar Petri-dish cultures cleared of bacterial colonics

('98). Wolfenden, Norris, and Forbes-Ross, F. W. A preliminary note on the action of the Roentgen rays upon the growth and activity of bacteria and micro-organisms. The Lancet, London, June 25, 1898, pp. 1,752-1,753.

Bacillus prodigiosus on potato was exposed to the rays for one hour on several occasions. Growth was much greater than in the control tubes, and more pigment was formed.

('98). Rieder, H. Weitere Mittheilung über die Wirkung der Röntgenstrahlen auf Bacterien sowie auf die menschliche Haut. Münch. med. Wochenschr., 45 Jahrg, 1898, pp. 773-774.

('01). Caspari, W. Ueber die bacterienschädigende Wirkung der Becquerelstrahlen. Nach in Gemeinschaft mit Priv. Doc Dr. Aschkinass ausgeführten Versuchen. Arch. ital. biol., Turin, T. xxxvi, 1901, p. 130.

('02). Rieder, Hermann Nochmals die bakterientodtende Wirkung der Röntgenstrahlen. Münchener med. Wochenschr., Bd. xlix, 1902, pp. 402-406.

('04). Prescott, S. C. The effect of radium rays on the colon bacillus, the diphtheria bacillus and yeast. Science, n. s., vol xx, Aug. 19, 1904, pp. 246-248.

"Radium rays have no effect upon fresh cultures of B. coli, B. diphtheria, or Saccharomyces cerevisiæ at a distance of one centimeter where the time of exposure is less than 90 minutes.

XXXII. Effect of High Pressure on Bacteria.

('75). Bert, P. Influence de l'air comprimé sur les fermentations. C. R. des sé. de l'Acad. des sci., Paris, 1875 T. lxxx, pp. 1,579-1,582.

('77). Bert, P. De l'emploi de l'oxygène à haute tension comme procédé d'investigation physiologique; des venins et des virus C. R. des sc. de l'Acad. des sci., Paris, 1877, T. lxxxiv, pp. 1,130-1,133.

('91). Schaffer et de Freudenreich. De la résistance des bactéries aux hautes pressions combinées avec une élévation de la température Annales de Microg., T. iv, 1891, pp. 105-119.

Milk subjected to a pressure of many atmospheres (75-90) for several hours at 45° to 65° C was not sterilized. High pressure for a week also failed to sterilize it.

('93). d'Arsonval et Charrin. Pression et microbes. La semaine médicale, 1893, T. xiii, p. 251. Rev. in Centralb. f. Bakt., Bd. xiv, 1893, p. 64. See also C. R. des sé. et mém. de la soc. de Biol., Paris, 20 mai, 1893, pp 532-533.

B. pyocyaneus, in fresh bouillon cultures, was subjected to a pressure of 50 atmospheres under carbon dioxide. All were dead inside of 24 hours. Even two hours' exposure interfered with the reproductive function, i. e., lessened the number of organisms capable of producing colonies, and in cultures made after four hours' pressure only traces of ability to form pigment remained. In cultures made after six hours exposure there was no formation of pigment, and generally no colonies when sown upon agar, but in one case there were a few.

('94). d'Arsonval and Charrin. See xxxiii.

('94). Roger. Action des hautes pressions sur les microbes. C. R. des sé. de l'Acad. des sci., T. cxix, Paris, p. 963.

Pressures of 1,000 to 3,000 atmospheres were tried without destroying the bacteria. Certain functions, however were destroyed, e. g., pathogenicity.

('97). Malfitano, G. Sul comportamento dei microorganismi all'azione dei gasi compressi. Boll. della Soc medico-chirurgica di Pavia, 1897. Rev. in Centralb. f. Bakt., xxiii Bd., 1898, pp. 233-236.

XXXIII. Action of Heat and Cold on Bacteria.

('75). Eidam, Eduard. Die Einwirkung verschiedener temperaturen und des Eintrocknens auf die Entwicklung von Bacterium termo Duj. Cohn's Beiträge z. Biol d. Pflanzen, Bd. I, Heft 3, pp. 208-224, Breslau, 1875.

('77). Frisch, A. Ueber den Einfluss niederer Temperaturen auf die Lebensfähigkeit der Bacterien. Sitzungsber. der K. Acad. der Wissensch. Wien. Math.-natur.-wissenschaften Classe, Mai, 1877, Bd. LXXV, III Abt., pp. 257-269.

('77). Tyndall, John. On heat as a germicide when discontinuously applied. Proc. Roy. Soc., London, vol. XXV, 1877, No. 178, pp. 569-570.

('79). Chamberland, Ch. Résistance des germes des certains organismes à la température de 100 degrés; conditions de leur développement. C. R. des sé. de l'Acad. des sci., T. LXXXVIII, 1879, pp. 659-661.

('82). Lebedeff, A. Contribution à l'étude de l'action de la chaleur et de la dessication sur la virulence des liquides septique et sur les l'organismes inférieurs. Archives de Physiol. normale et Path., Ser. II, T. X, pp. 175-204, 1882.

('84). Pictet, R., et Yung, E. De l'action du froid sur les microbes. C. R. des sé. de l'Acad. des sci., T. XCVIII, 1884, pp. 747-749.

('87). Prudden. See XLVI.

('87). Esmarch, E. Der Henneberg'sche Desinfector. Zeitschr. f. Hyg., Bd. II, 1887, pp. 342-368.

('88). Globig. Ueber einen Kartoffel-Bacillus mit ungewöhnlich widerstandsfähigen Sporen. Zeitschr. f. Hyg., Bd. III, 1888, pp. 322-332.

('88) Gruber, Max. Notiz über die Widerstandfähigkeit der Sporen von Bacillus subtilis gegen Wasserdampf von 100° C. Centralb. f. Bakt., 1888, Bd. III, pp. 576-577.

In six tubes of hay infusion inoculated with spores of B. subtilis, sealed by heating neck in flame, and then steamed ½ hour, there was an abundant growth of the hay bacillus in 36 hours at 37° C. Subsequently 21 samples of spores, dried on silk threads and exposed to streaming steam tor 2½ hours, in Thursfield's apparatus, grew readily; in 24 hours, at 35° C., there was a most luxuriant vegetation.

('88). Fischer, B. Bakterienwachstum bei 0° C. Centralb. f. Bakt., Bd. IV, 1888, pp. 89-92.

('90). Lustig, Alexander. Ein rother Bacillus im Flusswasser. Centralb. f. Bakt., Bd. VIII, 1890, pp. 33-40.

Lustig isolated a motile bacillus from river water, which grew from room-temperature (probably 15° C) to 60° C.

('92). Forster, J. Ueber die Entwickelung von Bakterien bei niederen Temperaturen. Centralb. f. Bakt., XII Bd., 1892, pp. 431-436.

The kinds of bacteria able to grow at 0° are not very numerous, but seem to be widely distributed, especially in water and on the surface and in the intestinal tract of fresh-water fish and salt-water fish.

('93). Pictet, Raoul. De l'emploi méthodique des basses températures en biologie. Archiv. d. sci phys. et nat., 3e Période, T. XXX, pp. 293-314, Genève, 1893.

Experiments with higher animals and plants, infusoria, microbes, diatoms With these two latter, excessive and prolonged cold gave negative results They were subjected to a temperature of minus 200°.

('93). Physalix. Influence de la chaleur sur la propriété sporogène du Bacillus anthracis. Abolition persistante de cette fonction par hérédité des charactères acquis. Arch. de physiol. normale et path., Paris, 1893, T. V, sér. 5, pp. 217-225.

('94). d'Arsonval et Charrin. Influence des agents cosmiques (électricité, pression, lumière, froid, ozone, etc.) sur l'évolution de la cellule bactérienne. Arch. de physiol. normale et path., 1894, T. VI, series 5, pp. 335-342.

('94). Waldo and Walsh. See XLVIII.

('94). Havemann. Ueber das Wachsthum von Mikroorganismen bei Eisschranktemperatur. (Inaug. Diss.) 8vo, 21 pp., Rostock, 1894.

Not seen.

('95). Sternberg, George M. What shall be the methods followed in determining the relation of bacteria to temperature? Jour. Am. Public Health Asso. Ann. vol. XX, 1895, pp. 411-414.

('95). Klepzoff, Const. Zur Frage über den Einfluss niederer Temperaturen auf die vegetativen Formen des Bacillus anthracis. Centralb. f. Bakt, XVII Bd, 1895, pp. 289-295.

Exposure to intense cold (average — 24° C.) for 12 days killed the anthrax organism in blood and various organs. Exposure for 25 days (at — 1° to — 24° C., average — 10.4° C.) killed agar cultures. The colonies in agar plates became less and less numerous as time passed No spores were present. Long exposure reduced the virulence.

('95). Miquel, P., et Lattraye. De la résistance des spores des bactéries aux températures humides égales et supérieures à 100°. Ann. de micr., T. VII, 1895, pp. 110-122, 158-170, and 205-218.

('99). Dannappel, Max. In wie weit ist die höhere Widerstandsfähigkeit der Bakteriensporen ein allgemeines Charakteristikum derselben gegenüber den vegetativen Spaltpilzformen? 8vo., pp. 27, Konigsberg, i. Pr., 1899, von E. Karg u. R. Manneck.

Some spores are said to show only a slight resistance to steam at 99° C. Of 25 species obtained from soils, decaying mixtures, milk, butter, etc., and said to be sporiferous, all but three were destroyed by exposure to steam at 99° C for 10 minutes and all but 8 by exposure for 5 minutes while 4 were killed by exposure for as short a time as 15, seconds, and two others by exposure for 1 minute. The names of these organisms are not given, so that the experiments cannot be duplicated, and in most cases it is not stated that the sporiferous nature of these bacteria was settled definitely by seeing the spores germinate. It is possible, therefore, that some of the extremely sensitive forms were not actually spore-bearing, but only gave microscopic appearances, which were interpreted as such. It is possible, also, that the spores were tested before they were fully matured. Only two of the very sensitive forms were examined critically, owing, it is said, to lack of time, and of one of these sensitive forms it is said: "A direct observation of the germination was not undertaken because the spore nature of the culture appeared unquestionable." Even heating for 1 to 3 minutes at 75° to 80° C destroyed this organism. Germination of the other was observed. The maximum temperature which could be endured in this case, for 1 minute, was 75° C. Both were green spores. Both were double stained by Moeller's method.

('99). Ravenel, M P. The resistance of bacteria to cold New York Medical News, vol. LXLIV, 1899 Also a separate, 5 pp. Rev. in Centralb. f. Bakt., XXVIII Bd, 1900, p. 751.

Tests in liquid air: B. diphtheriæ was alive at end of 30 minutes, B. typhi and B prodigiosus at the end of 60 minutes, B. anthracis after 3 hours.

('99) SMITH, THEOBALD. The thermal death point of tubercle bacilli in milk and some other fluids. Journal of Experimental Med., vol. IV, 1899, pp. 217-233 Rev. in Centralb. f. Bakt., XXVIII Bd., 1900, p. 409.

When embedded in the film on the surface of milk, Dr. Smith found the tubercle organism resisted a temperature of 60° C for an hour.

('99). KASANSKY, M. W. Die Einwirkung der Winterkälte auf die Pest- und Diphtheriebacillen. Centralb. f. Bakt., XXV Bd., 1899, pp. 122-124

These organisms withstood exposure for 6 months to severe temperatures. They were frozen all of the time for the first five months. From December 4 to 28, and again from February 13 to March 9, the maximum temperature was —10° to —23.4° C., and the minimum was —14° to —33.8° C.

('99). LEVIN. See XLII.

('99). MIRONESCO, THEODOR G. Ueber eine besondere Art der Beeinflussung von Mikroorganismen durch die Temperatur. Hygien. Rundschau, Jahrg. IX, 1899, pp. 961-964. Rev. in Centralb. f. Bakt., XXVII Bd., 1900, p. 86

('00). MEYER, J. Ueber Einwirkung flüssiger Luft auf Bakterien. Centralb. f. Bakt., XXVIII Bd., 1900, pp. 594-595

Anthrax spores and Staphylococcus pyog. aureus were tested. The exposure to the liquid air varied from 5 seconds to 15 minutes. Neither organism was killed. The temperature of liquid air is —190° to —220° C., according to Spiess, and —182° to —192° C. according to Macfadyen.

('00). SEDGWICK, W. T., AND WINSLOW, C. E. A. Experimental and statistical studies on the influence of cold upon the bacillus of typhoid fever, and its distribution. Jour. Bost. Soc. Med. Sci., vol. IV, No. 7, 1900, pp. 181-182. See also Centralb. f. Bakt., XXVII Bd., 1900, p. 684

30 to 60 per cent of the bacilli were destroyed in water during the first hour of freezing. After exposure for two weeks 90 per cent were destroyed. "The last two or three germs per thousand appear to be very resistant, some remaining after twelve weeks of freezing. The four races used showed constant individual differences in their susceptibility to cold. Alternate freezing and thawing was tested and found only slightly more destructive than continuous freezing." As several races of typhoid organism were tested, we may infer that ice is not very likely to communicate typhoid fever.

('00). PARK, WM. HALLOCK. A few experiments upon the effects of low temperature and freezing on typhoid bacilli. Jour. Bost. Soc. Med. Sci., vol. IV, No. 8, 1900, pp. 213-216.

Cultures were used from twenty different cases of typhoid fever. They behaved when frozen much as Sedgwick and Winslow's. On the average, at the end of twelve weeks' freezing only 0.05 of one per cent remained alive, i. e., 1,250 per cubic centimeter as against 2,500,410 per cubic centimeter at the beginning.

"At twelve weeks the bacilli in the ice from nine sources are all dead. Two more show no growth in 1 cc. The others contain from 80 to 11,000 in each cc. of ice. Only one, however, contains over 1,000 (culture 9). When typhoid bacilli are in feces, freezing does not exert so much of an effect. Thus typhoid and colon bacilli, originally 37,000 to a loopful of feces, were still 12,000 at the end of five weeks' exposure to a temperature ranging daily between zero and 28° F., and typhoid bacilli as well as colon were still abundant in the feces at nine weeks. It is a difficult matter to say for just how long a period ice made from infected water remains dangerous. The bacilli, even when few in number, are often vigorous and fully virulent, and, so far as I am aware, we are ignorant as to the number of bacilli required to start infection in man. The longer the infected ice remains frozen the less the number of pathogenic bacteria which remain alive in it."

('01). PARK, W. H. Duration of life of typhoid bacilli, derived from twenty different sources, in ice. Abstract of paper read at 2d meeting Soc. Am. Bacteriologists, Dec., 1900. Centralb. f. Bakt., 1 Abt., Bd. XXIX, 1901, pp. 444-445.

This describes the completion of an experiment already reported upon in part (see above). At the end of the twenty-second week of exposure the bacilli were dead in all the cultures of each one of the twenty races tested by freezing.

('01). D'ARSONVAL. La pression osmotique et son rôle de défense contre le froid dans la cellule vivante. C. R. des sé. de l'Acad. des sci., Paris, 1901, T. CXXXIII, pp. 84-86.

The fluid in the bacteria is probably not solidified, if the cell is not ruptured, owing to the enormous osmotic pressure in those small organisms. By lowering the osmotic tension the author thinks that any cell may be killed by cold.

('02). SCHMIDT-NIELSEN, SIGVAL. Ueber einige psychrophile Mikroorganismen und ihr Vorkommen. Centralb. Bakt., Abt. 2, Bd. IX, 1902, pp. 145-147.

('02). MACFADYEN, ALLEN, AND ROWLAND, SYDNEY. On the suspension of life at low temperatures. Abstract of paper read before Section K of the British Association, Belfast, 1902. Annals of Botany, vol. XVI, 1902, pp. 589-590

Various bacterial organisms were exposed from 20 hours to 7 days at —190° C. "These exposures did not produce any appreciable impairment in the vitality of the organisms, etc." Also 10 hours at —252° C. the temperature of liquid hydrogen had no appreciable effect on the vitality of the micro-organisms tested. Bacillus typhosus, B. coli communis, Staphylococcus pyogenes aureus and a Sacharomycete grew after exposure to liquid air for six months. "In no instance could any impairment of the vitality of the organisms be detected."

The objection to these statements is that quantitative determinations appear not to have been made, at least there is no mention of any. The writer of this review obtained a decided diminution of the number of viable bacteria in several species by exposure to liquid air for 20 hours.

('02). MACFADYEN, ALLEN. On the influence of the prolonged action of the temperature of liquid air on micro-organisms, and on the effect of mechanical trituration at the temperature of liquid air on photogenic bacteria. London, Proc. R. Soc., vol. LXXI, No. 468, Oct., 1902, pp. 76-77.

"The above experiments show that a prolonged exposure of six months to a temperature of about —190° C. has no appreciable effect on the vitality of micro-organisms."

The organisms tested were *B. typhosus*, *B. coli communis*, *Staphylococcus pyogenes aureus*, and a yeast. The triturated bacteria lost their luminosity.

('05). SMITH AND SWINGLE. See p. 83.

XXXIV. Thermophilic Bacteria.

('79) MIQUEL, P. Title? Bull. de la statistique municipale de Paris, Décembre, 1879

Not seen.

He discovered in the water of the Seine an immobile, rod-shaped Schizomycete capable of living and developing at the temperature of 70° C.

('81) VAN TIEGHEM, PH. Sur des bactériacées vivant à la température de 74° C. Bull. Soc. bot. de France, T. 28, 1881, pp. 35-36

This author cultivated several species of thermophilic bacteria at 70° C., and some at higher temperatures.

('81) MIQUEL. Thermobacteria. Annuaire de l'Observatoire de Montsouris, pour 1881, p. 464.

('86). CERTES, A., ET GARRIGOU. De la présence constante de micro-organismes dans les eaux de Luchon, recueillies au griffon à la température de 64°, et de leur action sur la production de la barégine. C. R. des sé. de l'Acad. des sci., T. CIII, 1886, pp. 703-706.

('88). GLOBIG. Ueber Bakterien-Wachsthum bei 50 bis 70° Zeitschr. f. Hyg., Bd. III, 1888, pp. 294-321. Rev. in Centralb. f. Bakt, Bd. III, 1888, pp. 366-368.

Globig obtained 30 sorts of bacteria which grew on potato at 58° C. At 68° only a few of them continued to grow. At 70° C. there were only scattering colonies, and at higher temperatures there was no growth. These organisms were not pathogenic to mice. As a rule, growth began at about 50° C, i. e., about 13 degrees above blood-heat One would not grow at 37° C. or 50° C, but grew at 60°. One grew all the way from 15° or 20° C. to 68° C. Spores were often formed in 24 hours. None of these were from feces or sewage. Most were bacilli (rods).

('88) MIQUEL, P. Monographie d'un bacille vivant à au-delà de 70° centigrades. Ann. de micro., 1888, T. 1, pp. 3-10.

This organism will not grow at temperatures under 40°, nor above 72° C. Its optimum temperature is 65° to 70° C.

('90). COHN, FERDINAND. Ueber Warmeerzeugung durch Schimmelpilze und Bakterien. Vortrag., Breslau, 1890.

('93). COHN, F. Ueber thermogene Bakterien. Ber. d. Deutsch. bot. Gesellsch., Bd. XI, 1893, Gen. Versamlungs-Heft, pp. 66-69.

Cohn found that when cotton wool waste was moistened, it reached a temperature of 67.2° C in 24 to 36 hours, and then slowly cooled (6 days) to the air-temperature When the same waste was sterilized there was no rise in temperature.

('94). MACFADYEN, ALLAN, AND BLAXALL, FRANK R. Thermophilic bacteria. Journal of Pathology and Bacteriology, vol. III, 1894, pp. 87-99. See also Br. Med. Jour., No. 1,760, 1894, p 644.

These authors obtained from garden soil an abundant growth of thermophilic bacteria on agar at 60° to 65° C, They also isolated these organisms from feces, sewage, sea-water, dirt of London streets, Thames water, Thames mud, straw, surface soil, and soil 5 feet 4 inches down These thermophilic bacteria are, therefore, most widely distributed. There were quite a variety of species—at least twenty. All were bacilli; all were spore-bearing. Some were actively motile. Active motility continued for three weeks in one hanging drop. The colonies developed very rapidly in agar-plates. Fifteen sorts were tested as follows: None of them grew at 22° or at 37° C. Horse-dung organisms grew at 40° to 42° C. Six sorts grew slowly at 50° to 52° C., and more abundantly at 60° to 65° C. Two grew first at 56°, and four refused to grow at 56°, but grew when the temperature was raised to 60° C. None would grow at 75°. The lower limit of growth for nearly all was 50° C. and the upper near 75° C. Boiling for ten minutes did not destroy these organisms. The optimum temperature for growth is said to be 60° to 65° C. These organisms did not ferment sugars and did not thrive on substrata containing sugars, these substances seeming to retard growth. One changed starch to sugar.

Query: How do these organisms exist in a climate as cold as that of Edinburg?

('95). RABINOWITSCH, LYDIA. Ueber die thermophilen Bakterien. Zeitschr. f. Hyg., Bd. XX, 1895, pp 154-164. Leipsic.

These thermophilic organisms were found in snow, in earth, in the dust of a street in Berlin They were very abundant in the water of the Spree (7,000 to 8,000 per cubic centimeter). They are abundant in the dung of horses and cows, and also more or less so in the excrement of goats, rabbits, guinea pigs, dogs, mice, doves, hens, ducks, parrots. They occur in the whole digestive tract of man, and are found in certain fish, frogs, and some other cold-blooded animals Miss R. also found them abundant in germinating barley in a brewery. They occur also in milk, even after it is boiled She isolated and studied 8 species. All produced spores None were pathogenic to mice or doves. The highest temperature at which any of them would grow was 75° C., and growth at this high temperature was slight. There was an abundant growth at 58° to 68° C., and the optimum is said to be 60° to 70°. They are very resistant to moist heat and also to dry heat. They were not killed by exposure to streaming steam for 5 to 6 hours. While growing best at high temperatures, these organisms can grow slowly facultative-anaerobically at 33° to 40° C., and the author thinks that they multiply inside warm-blooded animals. She found the temperature of dung-piles as high as 62° to 66° C.

('95). KARLINSKY, JUSTYN. Zur Kenntniss der Bacterien der Thermalquellen. Hygienische Rundschau, 1895, Bd V, pp. 685-689.

('96). TEICH, M Beitrag zur Kenntniss thermophiler Bacterien. Hygienische Rundschau, 1896, Bd. VI, No. 22, pp. 1,094-1,095.

('98). LAXA, O. Ueber einen thermophilen Bacillus aus Zucker-Fabriksproducten. Vorläufige Mittheilung. Zeitschr. f. Zuckerindustrie in Böhmen, Bd. XXII, 1898, p. 376.

Not seen.

('98). OPRESCU. Studien über thermophile Bakterien. Arch. f. Hyg., Bd. XXXIII, 1898, p. 164. Rev. in Centralb. f. Bakt., Bd. XXV, 1899, p. 360.

('99). TSIKLINSKY, MLLE. Sur les microbes thermophiles des sources thermales. Ann. de l'Inst. Pasteur, T. XIII, 1899, pp. 788-795. Bibliog. of 13 titles.

The author isolated five thermophilic organisms from hot springs. One form grew readily at 70° C

('99) MICHAELIS, GEORG. Beiträge zur Kenntniss der thermophilen Bakterien. Arch. f. Hyg., Bd. XXXVI, Hft 3, 1899, pp. 285-293. Rev. in Centralb. f. Bakt., XXVII Bd., 1900, p. 537.

Describes and names four new thermophilic organisms. All have three specific names, and one has four, to-wit: Bacterium thermophilus aquatilis liquefaciens aerobius.

('99). CAMBIER. Thermophilic bacteria as ferments, action on glucose. Rev. de phys. et de chim., 1899, p. 223.

Not seen.

('02). RUSSELL, H. L., AND HASTINGS, E. G. A Micrococcus, the thermal death limit of which is 76° C. Centralb. f. Bakt., 2 Abt., Bd. VIII, 1902, pp. 339-342, 1 plate.

('03). SETCHELL, WM. A. The upper temperature limits of life. Science, n. s., vol. XVII, 1903, pp. 934-937.

Hot springs were studied in three localities in California and in Yellowstone National Park. Author found only Cyanophyceæ and Bacteria in strictly thermal waters. The Cyanophyceæ were found at 65° to 68° C, and sparingly up to 75° to 77° C The bacteria were abundant at 70° to 71°, and occurred in considerable quantity at 82° C. and 89° C. "The temperature of 89° C. is the highest at which I have been able to find any organisms living." Care was taken to determine the temperatures in the exact places frequented by the organisms, so as to remove the objection that lies against many of the earlier observations

XXXV. Resistance to Dry Air.

('75). EIDAM. See XXXIII.

('92). MOMONT, L. Action de la dessication, de l'air, et de la lumière sur la bactéridie charbonneuse filamenteuse. Ann. de l'Inst. Pasteur, 1892, T. VI, pp 21-31.

('93) SWAN, ALLEN P. On the resisting vitality of the spores of Bacillus megaterium to the condition of dryness Annals of Botany, vol. VII, p. 153-154, 1893.

('94). WALLICZEK, HEINRICH. Die Resistenz des Bacterium coli commune gegen Eintrocknung. Centralb. f. Bakt., Bd. XV, 1894, pp. 940-950.

B. coli proved quite sensitive to dry air. The writer of this abstract has found great differences among bacteria e. g. B. tracheiphilus was killed by a few minutes' exposure on cover-glasses, while Bact. hyacinthi lived under similar conditions for more than a month. Jones found his Bacillus carotovorus to be even more sensitive than B. tracheiphilus. See following citations.

('95). SMITH, ERWIN F. Bacillus tracheiphilus, etc. Centralb. f. Bakt., 2 Abt., Bd. I, p. 370.

('97). MIQUEL, P. Sur la longévité des germes des bactéries dans les poussières et dans le sol. Ann. de micr., 1897, T. IX, pp. 199-207 and 251-259.

('01). JONES, L. R. A soft rot of the carrot, etc. 13th Ann. Rep. Vt. Agric. Exp. Sta. for 1900. Burlington, Vt., 1901. See p. 328 for reference to this subject.

('01). SMITH, ERWIN F. The cultural characters of Ps. hyacinthi, etc. Bull. 28, Div. Veg. Phys. and Path., U. S. Dep. Agr., Washington, D. C., 1901, p. 145.

XXXVI. Action of Acids and Alkalies.

('86). ABBOTT, A. C. The germicidal value of some of the vegetable acids. The Medical News, Phila., 1886, 9 Jan., pp. 33-34.

('92). DELBRUECK, M. Ueber das Verhalten der Cholerabacillen auf frischen Früchten, einigen Genuss- und Nahrungsmitteln. Sonderabdruck aus den Veröffentlichungen des Kaiserlichen Gesundheitsamte, 1892, No. 42, vom 19 October, Berlin. Verlag von Julius Springer, 1892, pp. 812-824.

The per cent of malic acid in many fruits is given. This varies from 0.13 (certain pears) to 2.65 (red currants). In the feebly acid fruits, the cholera bacilli were dead inside of 3 to 7 days; in the the tarter fruits they retained their vitality only for a period measured by hours. Usually they were dead in from 1 to 6 hours.

('92). SCHLUETER, G. Das Wachstum der Bakterien auf saurem Nahrboden. Centralb. f. Bakt., Bd. XI, 1892, pp. 589-598.

A dozen different bacteria were tested in "ordinary nutrient gelatin" and in fish glue, with addition of lactic acid, alum, tartaric acid, citric acid, acetic acid, and hydrochloric acid. Several organisms tolerated as much as 1 per cent of lactic acid, or 1 per cent tartaric acid, but their growth was slow and usually feeble. Several grew feebly in the presence of $\frac{1}{2}$ per cent alum. Six grew abundantly in gelatin acidified with citric acid, so that 8 cc. of the gelatin required for its neutralization 4 cc. of sodium carbonate water of the strength 5.3:1000. In fish glue containing 0.15 per cent acetic acid, several grew, but only feebly. Six grew in fish glue containing 0.075 per cent hydrochloric acid. The anthrax organism grew better with 0.2 per cent alum than on a neutral substratum.

('93). HESSE, W. Ueber den Einfluss der Alkalescenz des Nahrbodens auf das Wachsthum der Bakterien. Zeitschr. f. Hyg., Bd. XV, 1893, pp. 183-191.

('93). VOGES, O. Ueber das Wachstum der Cholerabacillen auf Kartoffeln. Centralb. f. Bakt., Bd. XIII, 1893, pp. 543-550.

Organism would not grow on potato as ordinarily prepared, but grew well at 37° C. (and more slowly at 20°) on the addition of a 2 to 3 per cent solution of sodium chloride. Nearly as good results were obtained with $\frac{1}{4}$ to $\frac{1}{2}$ per cent sodium carbonate solution. Growth was also obtained on potato with $\frac{1}{2}$ to $\frac{3}{4}$ per cent sodium hydrate solution.

('97). DEELEMAN, M. Der Einfluss der Reaktion des Nährbodens auf das Bakterienwachstum. Arbeit. aus dem Kaiserl. Gesundheitsamte, Bd. XIII, 1897, Heft 3. Rev. in Centralb. f. Bakt., XXII Bd., 1897, pp. 355-356.

('98). FERMI, CLAUDIO. Die Mineral- und organischen Sauren, die Alkali, die Alkaloide, das Jodkali und das arsensaure Kali zur Differenzierung der Mikroorganismen. Centralb. f. Bakt., Bd. XXIII, 1898, pp. 208-217 and 266-273.

Of the plant acids, oxalic was found to be the most deleterious to the Schizomycetes. The conclusions are given on p. 266 et seq.

XXXVII. Agglutination and Precipitation.

('96). WIDAL, FERNAND. Séro-diagnostic de la fièvre typhoïde. Bull. et mém. de la soc. méd. des hop. de Paris, 26 juin, 1896, pp. 561-566.

('97). WIDAL, F., ET SICARD, A. Etudes sur le séro-diagnostic et sur la reaction agglutinante chez les typhiques. Ann. de l'Inst. Pasteur, T. XI, 1897, pp. 353-432.

('97). FLEXNER, S. A recently discovered property of the blood serum in animals immune from certain diseases, and its application to the diagnosis of those diseases in human beings. Science (n. s.), vol. V, pp. 193-194, 1897.

('97). MALVOZ, E. Recherches sur l'agglutination du Bacillus typhosus par des substances chimique. Ann. de l'Inst. Pasteur, T. XI, 1897, pp. 582-590.

('98). NICOLLE, CHARLES. Recherches sur la substance agglutinée. Ann. de l'Inst. Pasteur, T. XII, 1898, pp. 161-191.

('99). BORDET, JULES. Le méchanisme de l'agglutination. Ann. de l'Inst. Pasteur, T. XIII, 1899, pp. 225-250.

('99). KRAUS, R. Ein Beitrag zur Kenntniss des Mechanismus der agglutination. Wiener Klin. Wochenschr. 1899, Jahrg. XII, pp. 1-4.

('99). GRUBER. Zur Theorie der Agglutination. Münch. med. Wochenschr., 1899, No. 41. Rev. in Centralb. f. Bakt., XXVII Bd., 1900, pp. 285-286.

('99). SABRAZÈS ET BRENGUES. Agglutinines chimiques. C. R. de la Soc. de biol., 1899, No. 35, p. 930. Rev. in Centralb. f. Bakt., XXVII Bd., 1900, p. 756.

('00). ZIKES. Ueber das Ausschleudern von Mikroorganismen unter Zuhilfenahme von Fällungsmitteln. Oesterr. Chemiker-Zeitung, 1900, No. 2. Rev. in Centralb. f. Bakt., XXVII Bd., 1900, p. 628.

('00). SMITH, R. GREIG. The flocculation of bacteria. The mechanism of agglutination. Proceedings of Linn. Soc. of New South Wales, 1900, Part 1, pp. 65-74, 75-83. Also a separate (issued Aug. 8, 1900).

('00). DURHAM, HERBERT E. Some theoretical considerations upon the nature of agglutinins, together with further observations upon Bacillus typhi abdominalis, Bacillus enteritidis, Bacillus coli communis, Bacillus lactis aerogenes, and some other bacilli of allied character. Jour. of Exp. Med., vol. V, pp. 353-388.

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('01). DODGE, CHARLES WRIGHT. A short method for the Widal test. Jour. of Applied Micro., vol. IV, 1901, p. 1,565. Also a separate.

('02). NEUFELD, F. Ueber die Agglutination der Pneumokokken und uber die Theorieen der Agglutination. Zeitschr. f. Hyg., 1902, Bd. XL, pp. 54-72.

('02). EISENBERG, PHILIPP, UND VOLK, RICHARD. Untersuchungen über Agglutination. Zeitsch. f. Hyg., 1902, Bd. XL, pp. 155-195.

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('02). JOOS, A. Untersuchungen über den Mechanismus der Agglutination. Zeitsch. f. Hyg., 1902, Bd. XL, pp. 203-230.

('02). SMITH, R. GREIG. Further remarks upon the mechanism of agglutination. Proc. Linnean Soc. of New South Wales, vol. XXVII, 1902, Part 1, pp. 66-72. Also a separate (issued Aug. 22, 1902).

('03). FLEXNER, SIMON. An aspect of modern pathology. Science, n. s., vol. XVIII, No. 444, 1903, pp. 3-15.

XXXVIII. Antiseptics and Germicides.

(See also XXXVI.)

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('74). DAVAINE, C. Recherches relatives à l'action des substances antiseptiques sur le virus de la septicémie. Gaz. med. de Paris, 1874, p. 44. Reprinted in l'Oeuvre de Davaine, Paris, 1889.

('75). LEWIN, L. Das Thymol ein Antisepticum und Antifermentativum. Virchow's Archiv, Bd. LXV, 1875, pp. 164-189. Polli's Annali di chimica applicata alla med. Milano, vol. LXII, 1876, pp. 321-324.

('77). POLLI, G. Sulle proprietà antifermentative dell'acido boracico e sue applicazioni alla terapia. Mem. Ist. Lomb., vol XIII, pp 453-468. Journ. de Pharm., et de chimie, T. XXVI, 1877, 4 sé., pp. 77-79.

('79). BOVET, V. Ueber die antiseptischen Eigenschaften der Pyrogallussaure. Journ. f. prakt. Chem. Neue Folge, Bd. XIX, pp. 445-461, 1879.

('80). PAVESI, C. Del solfato di potassa, e specialmente della sua proprietà antisettica, antifermentativa. Polli. Annali, vol LXXI, serie 3a, 1880, pp. 110-115.

('80). ENDEMANN, H. Boracic acid as a preservative. Chem. News, vol. XLI, pp. 152-153, 1880.

('80). SCHWARTZ, NICOLAI. Ueber das Verhalten einiger Antiseptica zu Tabacksinfusbacterien. Pharm. Zeitschr. f. Russland, Bd XIX, 1880, pp. 610-625, 641-658, 673-685.

Tested chloroform and found it of little worth. Experiments were made with [illegible] substances. The tabular summary is on pp 684-685. Picric acid heads the list for efficiency.

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('81). CHAPPUIS, E. Action de l'ozone sur les germes contenus dans l'air. Bull. de la Soc. chim. de Paris, 1 sém., n. s., T. XXXV, Paris, 1881, p. 290.

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('81). JALAN DE LA CROIX, N. Das Verhalten der Bakterien des Fleischwassers gegen einige Antiseptica. Arch. f. exper. pathol. u. pharm., 1881, Bd. XIII, pp. 175-255. Ber. d. deutsch chem. Gesellsch., Bd. XIV, pp. 2,835-2,838.

('81). GOSSELIN, L., ET BERGERON, A. Recherches sur la valeur antiseptique de certaines substances et en particulier de la solution alcoolique de Gaultheria. Arch. gén. de méd., Paris, 1881, vol. 1, VIII, sé., T. 7 (misprinted 6), 147 vol. de la collection, pp. 16-29.

('82). BERT, P., ET REGNARD, P. Action de l'eau oxygénée sur les matières organique et les fermentations. C. R. des sé. de l'Acad. des sci., T. XCIV, 1882, pp. 1,383-1,386.

('82). BURCQ, V. Sur l'action désinfectante et antiseptique du cuivre. C. R. des sé. de l'acad des sci., T. XCV, 1882, pp. 862-864.

Workers in copper escape both cholera and typhoid fever. It is said that there has not been a single death from either disease in the Sociéte de Bonaccord (copper, bronze and brass workers) since its establishment in 1819.

('82). SCHIEFFERDECKER, P. Ueber eine neue Injectionsmasse zur Conservirung der Leichen fur den Präparirsaal. Arch. f. Anat. u. Entwickelungsgesch., 1882, pp. 197-198.

('82). VULPIAN. Etudes expérimentales relatives à l'action que peut exercer le permanganate de potasse sur les venins, les virus et les maladies zymotiques. C. R. des sé. de l'Acad. des sci., T. XCIV, 1882, pp. 613-617. Jour. de Pharm. et de Chimie, T. VI, 5 série, 1882, pp. 100-104.

('86). UNNA, P. G. Ichthyol und Resorcin als Repräsentaten der Gruppe reduzierender Heilmittel. Hamburg, 1886. Unna's Dermatologische Studien, 2 Heft, pp. 1-85.

('88). NUTTALL, GEO. Experimente uber die bakterienfeindlichen Einflüsse des thierischen Körpers. Zeitschr. f. Hyg., Bd. IV, 1888, pp. 353-394, 1 plate.

('88). SALKOWSKI, E. Ueber die antiseptische Wirkung des Chloroformwassers. Deutsche medicin. Wochenschrift, 1888, Bd. XIV, pp. 309-311.

('88). LOEW, O. Physiologische notizen über Formaldehyd. München. med. Wochenschr., 1888, Bd. XXXV, pp. 412-413. Physiol. Ges. Munchen (1-6), 1888, pp. 39-41. Rev. in Ch. Centralb., 1889, LX Jahrg., Bd. I, p. 90.

('88). BEHRING. Ueber Quecksilbersublimat in eiweisshaltigen Flüssigkeiten. Centralb. f. Bakt., 1888, Bd. III, pp. 27-30 and 64-66.

('89). FRAENKEL, CARL. Die Einwirkung der Kohlensäure auf die Lebensthätigkeit der Mikroorganismen. Zeitsch. f. Hyg., Bd. V, 1889, pp. 332-362.

('90). ALTEHOEFER. Ueber die Desinfectionskraft von Wasserstoffsuperoxyd auf Wasser. Centralb. f. Bakt., 1890, Bd. VIII, pp. 129-137.

('90). KIRCHNER, M. Untersuchungen über der Einwirkung des Chloroforms auf die Bakterien. Zeitschr. f. Hyg., Bd. VIII, pp. 465-488, 1890.

('90) Sonntag, Hermann. Ueber die Bedeutung des Ozons als Desinficiens. Zeitschr. für Hyg., 1890, Bd. VIII, pp 95-136.

('91). Tizzoni, Guido, u. Cattani, G. Ueber die Widerstandsfahigkeit der Tetanusbacillen gegen physikalische und chemische Einwirkungen. Archiv. f. exper. Path. u. Pharm., 1891, Bd. XXVIII, pp 41-60.

('91) Froelich, O. Ueber das Ozon, dessen Herstellung auf elektrischem Wege und dessen technische Anwendungen, insbesondere in der Gesundheitstechnik. Gesundsheits-Ingenieur, 1891, No. 16, pp. 543-551.

('91) Froelich, O. Ueber das Ozon, dessen Herstellung auf elektrischem Wege und dessen technische Anwendungen. Electrotechnische Zeitschr., 1891, 12 Jahrg., pp. 340-344.

Contains short paragraph on physiological action of ozone. Bacteria living in water are killed "sämmtlich." No experiments with pathogenic bacteria. It is still a question whether bacteria in the air are killed.

('91) Gerlach, Val. Ueber Lysol. Zeitschr. f. Hyg., Bd. X, 1891, pp 167-196. Also a separate.

Lysol is more active than carbolic acid or creolin. The hands may be disinfected in a 1 per cent. solution without soap. Surgical instruments may be sterilized in ¼ per cent solution without the least injury. Walls may be disinfected in a 3 per cent solution. It is to man the least poisonous of the antiseptics of its class.

('91) Fischer. See XI.

('92) Schlueter. See XXXVI.

('92). Richtet, Ch. De l'action de quelques sels métaliques sur la fermentation lactique. C. R. des sé. de l'Acad. des sci., T. CXIV, 1892, pp. 1,494-1,496.

('92) Ohlmueller. Ueber die Einwirkung des Ozons auf Bakterien. Arbeiten aus dem Kaiserl. Gesundheitsamte, Bd. VIII, 1892, Heft 1, pp. 229-251.

Ozone in water is less effective as a germicide in proportion as the water contains more and more dead organic matter. It is not adapted to the disinfection of rooms.

('92) Delbrueck. See XXXVI.

('92). Hammer, Hans. Ueber die desinficirende Wirkung der Kresole und die Herstellung neutraler wässriger Kresollosungen. II Mittheilung. Arch. f. Hyg., Bd. XIV, 1892, pp. 116-134.

('92) Aronsohn, Hans. Ueber die antiseptischen Eigenschaften des Formaldehyde. Berl. klin. Wochenschr., 1892, Bd. XXIX, No. 30, pp 749-751.

('92). Berlioz, F., and Trillat, F. Sur les propriétés des vapeurs du formal ou aldéhyde formique. C. R. des sé. de l'Acad. des sci., 1892, T. CXV, pp. 290-292.

('92) Hankin, E. L'action bactéricide des eaux de la Jumna et du Gange sur le microbe du choléra. Ann. de l'Inst. Pasteur, T. X, pp 511-523.

Cholera does not descend the rivers in India. Bacteria are much rarer in these rivers than in European rivers. The filtered, unboiled river water has a decided bactericidal action on the cholera organism. When boiled the water lost its germicidal property.

('93) de Christmas, J. Sur la valeur antiseptique de l'ozone. Ann. de l'Inst. Pasteur, T. VII, 1893, pp. 776-780.

('93) Schild. See XVIII.

('93). Voges. See XXXVI.

('93) Green. Ueber den Werth der Kupfersalze als Desinfektionsmittel. Zeitschr. f. Hyg., Bd. XIII, 1893, pp. 495-511.

States that copper salts have considerable value as germicides, especially the soluble ones. Cuprum bichloratum is considered most valuable. This is the only copper salt that is sufficiently active in solutions containing much albumen. For the treatment of wounds, copper bichlorate is much better than copper sulphate.

('93) Loew, Oskar. Ein natürliches System der Giftwirkungen. Munchen, 1893, Wolff und Luneburg, pp VIII, 136. Rev. in Centralb. f. Bakt., 1893, Bd. XIV, p. 234.

('93) Gruber, Max. Ueber die Loslichkeit der Kresole in Wasser und über die Verwendung ihrer wassrigen Losungen zur Desinfektion. Arch. f. Hyg., Bd. XVII, 1893, pp. 618-625.

('94). Dieudonne. See XXIX.

('94). d'Arsonval et Charrin. See XXXIII.

('94). Abel. See XVIII.

('94) Miquel. De la désinfection des poussières sèches des appartements; and Contribution nouvelle à l'étude de la désinfection par les vapeurs d'aldéhyde formique. Ann. de micr., T. VI, 1894. See pages 257, 305, 306, 520, 588, and 621.

('94). Pottevin, Henri. Recherches sur le pouvoir antiseptique de l'aldéhyde formique. Ann. de l'Inst. Pasteur, T. VIII, 1894, pp. 796-810.

('94) Bolton, Meade. The effect of various metals on the growth of certain bacteria. Internat. Med. Mag., December, 1894, pp. 812-822. Also a separate. Reviewed in Am. Nat., Oct., 1895, p. 933.

('94). Schilow, P. F. Ueber den Einfluss des Wasserstoffsuperoxydes auf einige pathogene Mikroorganismen. St. Petersb. med. Wochenschr., 1894, No. 6. Rev. in Centralb. f. Bakt., Bd. XVI, 1894, pp 42-43.

Cholera bacteria were destroyed in 3 minutes in 1:200; in 1:300 they were alive after 1 hour. Typhoid bacilli, in 1:100 to 1:200, were killed in 10 minutes; in 1:1000, after 1 hour. Anthrax spores, in 14 per cent solution were killed in less than 3 minutes, a 2 per cent solution killed them in less than one hour, a 1 per cent solution did not kill in 1 hour. Staphylococcus pyogen. aureus, from cultures 1 day old, was killed in 1:100 in less than 10 minutes; in 1:200 it required more than 15 minutes. Diplococcus pneumoniæ does not grow in bouillon to which hydrogen peroxide has been added in 1:10,000 to 1:18,000. Solutions of 1:200 destroyed a one-day old culture in 15 minutes.

('94). Walliczek, Heinrich. Die baktericiden Eigenschaften der Gerbsäure (Tannin der Apotheken). Centralb. f. Bakt., Bd. XV, 1894, pp 891-894.

Tables showing effect of various per cents of tannin on B. coli, B. anthracis, and Staphylococcus aureus.

('95). Burckhard, G. Zwei Beitrage zur Kenntnis der Formalinwirkung. Centralb. f. Bakt., XVIII Bd., 1895, pp 257-264.

Twenty titles are cited at the end of this paper.

('95). van Ermengem, E. De la stérilization des eaux par l'ozone. Ann. de l'Inst. Pasteur, T. IX, 1895, pp. 673-709. Rev. in Centralb. f. Bakt., Bd. XIX, 1896, pp 836-838, 2 figs.

Van Ermengem's report is favorable.

('95). Schepilewsky, Eugen. Formaldehyd als Desinfektionsmittel. (Dissert.) St. Petersburg, 1895. (Russisch.) Rev. in Centralb. f. Bakt., Bd. XIX, 1896, pp. 704-706.

('95). Goriansky, G. J. Sur la désinfection des crachats phtisiques et des cultures tuberculeuses par les solutions alcalines de goudron et de vinaigre de bois. Arch. des sci. biol., pub. par l'Inst. imp. de méd. exp. à St Pétersburg, Tome 3, 1895, pp. 148-166.

Wood-vinegar is a very energetic disinfectant. In quantity equal to the volume of sputum, and acting for 6 hours, it was found entirely efficient Exposure of 4 hours is not sufficient in some cases to destroy B. tuberculosis in sputum. In pure culture the organism is destroyed by exposure for 1 hour to this acid.

('95). d'Arsonval. Sur la production de l'ozone concentré et sur ses effets bactéricides. C. R des sé. et mém. de la soc. de biol., Paris, 10 sé , T. 11, 1895, pp. 500-502.

The writer's experiments were negative, and he is very skeptical as to germicidal power of ozone.

('95). Fischer. See xl.

('96). Walter, K. Zur Bedeutung des Formalins, bezw. Formaldehyde als Desinfektionsmittel. Zeitschr. f. Hyg., Bd. xxi, 1896, pp. 421-451. Rev. in Centralb. f. Bakt., xx Bd., 1896, p. 280.

('97). Iwanoff, W. A. Zur Frage über das Eindringen der Formalindämpfe in die organischen Gewebe. Centralb. f. Bakt., xxii Bd., 1897, pp. 50-58.

Formalin vapor did not penetrate rapidly into the depths of the tissues tested (livers of rabbits and guinea pigs).

('97). Weyland, J. Desinfektionswirkung und Eiweissfällung chemischer Körper. Centralb. f. Bakt., xxi Bd., 1897, pp. 798-802.

('97). Fuerbringer und Freyhan. Neue Untersuchungen über die Desinfektion der Hände. Deutsche med. Wochenschr., 1897, No. 6. Rev. in Centralb. f. Bakt., xxi Bd., 1897, pp 708-710.

Authors recommend alcohol. A 2 per cent solution of mercuric chloride is still better. Both may be used, the latter preceded by the former.

('97). Podgorny, K. M. Effect of iodine on pathogenic bacteria. Thesis of St. Petersburg, No. 36, 1897, pp. 74.

('97). Schumburg. Ein neues Verfahren zur Herstellung keimfreien Trinkwassers. Deutsche med. Wochenschr., Bd. xxiii, 1897, No. 10, pp. 145-146.

Bromide treatment.

('98). Minervini, Rafael. Ueber die baktericide Wirkung des Alkohols. Zeitschr. f. Hyg., Bd. xxix, 1898, pp. 117-148. Bibliography of 18 titles.

Ethyl alcohol has only a weak bactericidal action. It is most active in concentrations of 50 to 70 per cent. Alcoholic solutions of antiseptic substances are less active than water solutions.

('98). Schultz, N. De l'action des antiseptiques sur le bac. pestis hominis et de la désinfection d'effects et de locaux contaminés par la peste bubonique. Arch. des sci. biol. publiées par l'inst. impér. de méd expér. à St. Petersbourg, T. vi, 1898, pp. 307-426, 1 plate.

The appearance of the bacteria subjected to the antiseptics, as shown on the plate, strongly suggests the appearance of organisms in old cultures, viz.: involution forms, and the two phenomena may be due to the same cause, the involution forms arising from the harmful action of products excreted by the bacteria, or arising from the action of substances developed in the media as the result of bacterial occupation

('98). Fluegge, C. Die Wohnungsdesinfektion durch Formaldehyd. Zeitschr. f. Hyg., Bd. xxix, 1898, pp. 276-308

('98). Fermi. See xxxvi.

('98). Popoff, S. P. Vergleichende Studien über die desinfizierende Wirkung reiner Sublimatlösungen und Kombinationen derselben mit anderen Desinficientien. (Diss.) St. Petersburg, 1898. Rev. in Centralb. f. Bakt., xxv Bd., 1899, pp. 331-332.

The power of 1 per cent sol. mercuric chloride is increased very decidedly by addition of 1 per cent hydrochloric acid, or 1 per cent phenol, and by the addition of 2 per cent phenol a still more effective mixture is obtained. Addition of 1 to 2 per cent NaCl weakened the effect of the 1 per cent sublimate solution on some organisms, but increased it on others.

('98). Lucas-Championnière. Sur la valeur antiseptique de l'eau oxygénée. Bull. de l'acad. de méd., 1898, T. xl, série 3, Paris, pp. 599-617.

('99). Marmier et Abraham. La stérilisation industrielle des eaux potables par l'ozone. Rev. d'hyg. et de Police Sanitaire, Paris, 1899, T. xxi, pp. 540-554.

Great things are claimed for this method. Only some specimens of Bacillus subtilis are said to have escaped destruction, and of these only one individual for each 15 cc. of water treated with a concentration of ozone equal to 6 milligrams per litre of air.

('99). Stadler, Eduard. Ueber die Einwirkung von Kochsalz auf Bakterien, die bei den sogenannten Fleischvergiftungen eine Rolle spielen. Arch. f Hyg., Bd. xxxv, 1899, pp. 40-82. Rev. in Centralb. f. Bakt., xxvi Bd., 1899, p 411.

B. coli, B. morbificans bovis, and B. enteritidis grew in bouillon with 7 per cent sodium chloride. During the first 2 to 3 days they were injured, but after that they made a luxuriant growth. The toleration limit for B. coli and B. enteritidis is between 7 and 8 per cent of NaCl, and that of B. morbificans bovis between 8 and 10 per cent. The writer of this abstract found certain plant bacteria much more sensitive to salt, e. g Ps hyacinthi was restrained by 1.5 per cent.

('99). Bliss, C. L., and Novy, Fr. G. Action of formaldehyde on enzymes and on certain proteids. The Jour. Exp. Medicine, vol. iv, 1899, pp. 47-80.

('99). Weyl, Th. Keimfreies Trinkwasser mittels Ozon. Centralb. f. Bakt., xxvi Bd., 1899, pp. 15-32, with 1 fig.

"Ozone is a specific bacterial poison." It is recommended for sterilizing drinking water.

('99). Koch, E., and Fuchs, G. Ueber den antibakteriellen Wert des Acroleïn. Centralb. f. Bakt., xxvi Bd., 1899, pp. 560-563.

Acroleïn is a substance related to formaldehyd. In 0.25 to 0.5 per cent solutions it proved more effective than formaldehyd on a number of non-sporiferous organisms.

('99). Calmette, A Rapport sur la stérilization industrielle des eaux potables par l'ozone. Ann. de l'Inst. Pasteur, 1899, T. xiii, pp. 344-357.

A favorable report on sterilization of water by ozone by a committee, of which Calmette was secretary. They recommended the system of Marmier and Abraham for the city of Lille.

('99) Roux et Calmette. Sterilization of water by ozone. Rapport présénté à la municipalité de Lille, février, 1899.

Not seen.

('99). Marpmann. Die bakterieide Wirkung des Fluornatriums und der Nachweis desselben in Nahrungsmitteln. Centralb f. Bakt., Bd. xxv, 1899, pp. 300-311.

Considers sodium fluoride a valuable disinfectant for all bacteria in nutrient media. Sodium fluoride is said to be scarcely more harmful to man than common salt In beer wort in doses of 1 gram per litre it inhibits the growth of bacteria without lessening that of the yeast

('00). Otto Sterilization of water by ozone. Bulletin de la Société des ingénieurs civils de France, février, 1900.

('01). Mayer, Eugen, und Wolpert, Heinrich Beiträge zur Wohnungsdesinfektion durch Formaldehyd: i. Die zweckmässigste Form des Verdampfungsapparats. ii. Einfluss der Temperatur auf die Desinfektionswirkung iii. Verstärkung der Desinfektionswirkung durch künstliche Luftmischung (Vorl. Mitt.). Hyg. Rdsch., Berlin, xi Jahrg., 1901, pp. 153-158.

('01) Smith, Erwin F. Growth of bacteria in the presence of chloroform and thymol. Science, n. s., vol. xiii, p 327, March 1, 1901. See also Jour. Boston Soc. Med. Sci., vol. v, p. 375, and Centralb. f. Bakt., 1901, 1 Abt., Bd. xxix, pp. 445-446.

('01). Hess, Otto. Der Formaldehyd. Seine Darstellung, Eigenschaften, und seine Verwendung als Konservierungs-therapeutisches und Desinfektionsmittel mit besonderer Berücksichtigung der Wohnungsdesinfection. Marburg, N G Elwert, 2 Aufl, 1901, pp. iv, 120.

('02) Rolly. Zur Analyse der Borax- und Borsäurewirkung bei Fäulnissvorgängen, nebst Studien über Alkali- und Säureproduktion der Faulnissbakterien. Arch. f. Hyg., Bd. xli, 1902, pp. 348-405.

('02). Prescott, Samuel C Antiseptics and their use in the preservation of food. Technology Quarterly. Vol. xv, 1902, pp. 335-342.

('02). Hill, Hibbert W., and Rickards, Burt R. Notes on formaldehyd Proc. Am Pub. Health Asso., 30th Ann. Meeting, New Orleans, Dec., 1902. Also a separate, pp. 12.

('02). Schüder. Entgegnung auf die Schumburg'sche Arbeit: "Das Wasserreinigungsverfahren mit Brom" und die Arbeit von A Pfuhl: "Zu den Schüder'schen Prüfungsversuchen des Bromverfahrens nach Schumburg." Zeitsch. f. Hyg., Bd. xxxix, 1902, pp. 532-539

('02). Schumburg, Wilhelm. Zu der Schüder'schen Entgegnung bezüglich des Bromverfahrens zur Trinkwasser-Reinigung. Zeitsch. f. Hyg., Bd xl, 1902, pp. 199-202.

('02). Green, A. B. The disinfectant action of chloroform and various other substances on the specific and extraneous micro-organisms of vaccine. Rep. Med. Off. Loc. Gov., London, 1902, p. 639-663.

('02). Engels, Eugen Das Schumburg'sche Verfahren der Trinkwasserreinigung mittels Brom Centralb. f. Bakt., Abt 1, Bd. xxxi, Originale, 1902, pp. 651-670.

('02) Konrádi, Dániel. Ueber die baktericide Wirkung der Seifen. Arch. f. Hyg., Bd. xliv, 1902, pp. 101-112.

Recommends a resorcin soap.

('02). Mayer, Eugen, und Wolpert, Heinrich. Ueber die Verfahren und Apparate zur Entwicklung von Formaldehyd für die Zwecke der Wohnungsdesinfektion. Arch. f. Hyg., Bd. xliii, 1902, pp. 157-169.

('02). Cohn, Ernst. Ueber den antiseptischen Wert des Argentum colloidale Credé und seine Wirkung bei Infektion. Diss. Königsberg i. Pr. (Druck v. M Hiller), 1902, p. 57.

('03). Rickards, Burt Ransom. A comparison of some of the more common liquid disinfectants Jour. Mass. Asso. of Boards of Health, vol. xiii, No. 3, Oct., 1903, pp. 70-76.

('03). Freer, Paul C., and Novy, Fredr. G. On the organic peroxides Vaughan Quarter Century Book, pp. 63-127 Ann Arbor, 1903

"Acetyl and benzoyl hydrogen peroxides are extremely germicidal, and easily rank with the most active disinfectants * * Hydrogen peroxide is considerably weaker than these organic peroxides The activity of the peracids and of hydrogen peroxide is not due to active oxygen, but is probably due to the acid ions."

('03) Rideal, Samuel. Disinfection and the preservation of food, together with an account of the chemical substances used as antiseptics and preservatives. 3d ed., London, Sanitary Pub. Co., Ltd; New York, John Wiley & Sons, 1903, pp 494

('04). Konrádi, Dániel Weitere Untersuchungen ueber die bakterizide Wirkung der Seifen Centralb. f. Bakt., 1 Abt., Originale, xxxvi Bd., 1904, pp. 151-160.

St. Láceleau soap is actively bactericidal.

('05) Kraemer, Henry. The Oligodynamic Action of Copper Foil on Certain Intestinal Organisms. Proc. Am. Phil. Soc., vol. xliv, pp. 51-65. Phila., 1905 Also a separate.

XXXIX. Chemotropism, Thermotropism, Geotropism, Contact-Irritation, Etc.

('84). Pfeffer, W. Lokomotorische Richtungsbewegungen durch chemische Reize. Untersuchungen aus dem bot. Institut Tübingen, 1 Bd., 1884, pp. 363-482

The chapter on Spaltpilze begins on p. 449.

('86) Dubois, R. Influence du magnétisme sur l'orientation des colonies microbiennes. C. R. des sé et mém. de la soc. de biol., Paris, 1886, 8 sé, T. iii, pp. 127-128.

('88). Pfeffer, W. Ueber chemotaktische Bewegungen von Bakterien, Flagellaten und Volvocineen. Untersuch. a. d. bot. Inst. zu Tübingen, 1888, Bd. ii, Heft 3, pp. 582-661.

('90). Ali-Cohen, Ch. H. Die Chemotaxis als Hülfsmittel der bakteriologischen Forschung. Centralb. f. Bakt., viii Bd., 1890, pp. 161-167.

('93). Boyce and Evans. Upon the action of gravity on Bacterium Zopfii. Communication made to the Royal Society, Feb., 1893. Rev. in Centralb. f. Bakt., Bd. xv, 1894, pp. 568-569

('93). Roth, A. Ueber das Verhalten beweglicher Mikroorganismen in strömenden Flüssigkeiten. Deutsch. med. Wochenschr., 1893, No. 15, pp. 351-352

In streaming fluids this author observed in motile bacteria a decided tendency to move against the current.

('94). Miyoshi, Manabu. Ueber Chemotropismus der Pilze. Botanische Zeitung, 1894, Hft 1, col. 1-28, with 1 table.

Deals only with fungi

('94) Beyerinck, M. W. Ueber Thermotaxis bei Bakterium Zopfii. Centralb. f Bakt, Bd xv, 1894, p. 799

Refers to thermotaxis the movements of the threads of Bact Zopfii, which Boyce and Evans supposed to be due to geotropism.

('01) Jennings, H. S., and Crosby, J. H. Studies on reactions to stimuli in unicellular organisms. vii. The manner in which bacteria react to stimuli, especially to chemical stimuli. Am Jour. of Physiol., vol. vi, 1901, pp. 31-37. Also a separate.

The movement of unicellular organisms toward or away from chemical substances is said to be due to a "motor reflex" comparable to that of the ciliate infusoria, and not to chemotaxis, as stated by Pfeffer and others. These conclusions agree with those of Rothert.

('01) Rothert, W Beobachtungen und Betrachtungen uber tactische Reizerscheinungen. Flora oder Allgemeine Botanische Zeitung, 88 Bd, 1901, Hft. III, pp. 371-421.

('02). Clark, Judson F. On the toxic properties of some copper compounds with special reference to Bordeaux mixture Botanical Gazette, vol. xxxiii, 1902, pp 26-48, 7 figs.

Controverts Miyoshi on chemotropism.

('03). Rothert, W. Ueber die Wirkung des Aethers und Chloroforms auf die Reizbewegungen der Mikroorganismen. Jahrb f. Wiss. Bot., Bd. xxxix, 1903, pp. 1-70

The variability in sensitiveness of the same organism at different times was most disturbing. "Material which to-day is strikingly chemotactic or phototactic, may be to-morrow unusable. Especially striking and perplexing was the behavior of *Spirillum undula*, this classical object for chemotaxis and osmotaxis, with which Pfeffer made his celebrated investigations, and with which in former years I confirmed the experiments of Pfeffer."

XL. Osmotic Pressures.

('91). Wladimiroff, Alexander. Osmotische Versuche an lebenden Bakterien Zeitschr. f. physikalische Chem., Bd vii, 1891, pp. 529-543.

('91). Wladimiroff, Alexander. Biologische Studien an Bakterien. i. Uber das Verhalten beweglicher Bakterien in Losungen von Neutralsalzen. Zeitschr. f. Hyg., 1891, Bd. x, pp. 89-110.

('91). Fischer, Alfred. Die Plasmolyse der Bakterien. Ber. uber die Verhandlungen d. K. sächs. Ges d. Wissenschaften. Mathem-phys Classe, Leipzig, 1891, Bd. xliii, pp 52-74, 1 plate.

Plasmolysis was either definitely established or rendered extremely presumptive (smaller forms) for 17 kinds of bacteria. The concentration which induced it varied in most cases from 0.5 to 5 per cent. In a few cases the least concentration which would induce plasmolysis was not determined Generally strengths of 1 to 2 per cent sodium chloride were sufficient. "Die untere Grenze liegt fast fur alle Bacterien bei 1 per cent oder 0.75 per cent NaCl."

('95). Fischer, Alfred. Neue Beobachtungen uber Plasmolyse der Bakterien in Untersuchungen uber Bakterien Jahrb. f. wissensch. Bot., Berlin, 1895, Bd xxvii, pp. 1-34.

('01). d'Arsonval. See xxxiii.

XLI. Chemical Analysis of Bacteria.

('79). Nencki, M., und Schaffer, F. Ueber die chemische Zusammensetzung der Faulnissbakterien. Jour. f. Praktische Chemie, neue Folge, Bd. xx, 1879, pp. 443-466, 1 fig., 1 plate. Also a separate.

('81). Schaffer, F. Zur kenntniss des Mykoproteins. Journal f. Prak. Chemie, neue Folge, Bd. xxiii, 1881, pp. 302-304.

('86). Brown, Adrian J. On an acetic ferment which forms cellulose. Journal Chem. Soc. Trans., London, vol. xlix, pp. 432-439.

('87). Brown, Adrian J. Note on the cellulose formed by Bacterium xylinum. Journ. Chem. Soc., London, Trans., vol. li, 1887, p. 643.

('87). Vincenzi, Livio. Ueber die chemischen Bestandteile der Spaltpilze Zeitschr. f. physiolog. Chemie, 1887, Bd. xi, pp. 181-183

('88). Hammerschlag. Ueber bacteriologisch-chemische Untersuchung der Tuberkelbacillen. Verhandlungen der Schweizerischen Naturf. Gesselsch. in Solothurn, August, 1888, 71 Jahresversammlung, pp. 85-86.

('93). Cramer, E. Die Zusammensetzung der Bakterien in ihrer Abhangigkeit von dem Nahrmaterial. Arch. f. Hyg., Bd. xvi, Heft 2, 1893, pp. 151-195.

('93). Nishimura, Toyosaku. Untersuchung über die chemische Zusammensetzung eines Wasserbacillus. Arch. f. Hyg., Bd. xviii, 1893, pp. 318-333.

('93). Dreyfuss, Isidor. Ueber das Vorkommen von Cellulose in Bacillen, Schimmel- und anderen Pilzen. Zeitschr. f. physiol. chem., Bd. xviii, 1893, pp 358-379.

The conclusion is that cellulose occurs in hay bacilli and in pus bacilli.

('95) Cramer, E. Die Zusammensetzung der Cholerabacillen. Arch. f. Hyg., Bd xxii, 1895, pp. 167-190.

('98). de Schweinitz, E. A., and Dorset, Marion. The mineral constituents of the tubercle bacilli. Centralb. f. Bakt., Bd. xxiii, 1898, pp. 993-995.

('02). Kresling, K. I. De la substance grasse des bacilles de la tuberculose. Arch. des sci. biol., publiées par l'inst. imp. de méd. expér. à St. Pétersbourg, T. ix, 1902, pp. 359-376

XLII. Distribution of Bacteria—Geographical and Altitudinal.

(Deserts, mountains, arctic regions, sea air, depths of the sea, deep wells, surface soils, air at the earth's surface, and at higher levels.)

('81). Miquel, P. Sur le dosage des bactéries dans les poussières et dans le sol. Bull. Soc Bot. de France, 1881, T xxviii, sér. 3, pp. 44-51.

('82). Tyndall, John. Essays on the floating-matter of the air in relation to putrefaction and infection New York, D. Appleton & Co., 1882, pp. xix, 338.

('83) Miquel, P Les organismes vivants de l'atmosphère, pp. viii, 310. Paris, 1883. Gauthier-Villars.

('83) Miquel, P. Nouvelles recherches sur les bactéries atmosphériques effectuées à l'observatoire de Montsouris. Ann. de l'observatoire de Montsouris pour l'an 1883, pp. 301-437

('83). Miquel, P. De la pureté en microbes de l'air des montagnes et de quelques districts de la Suisse. La semaine médicale, 1883, pp. 274-276.

('83) Giacosa, Piero. Studii sui corpuscoli organizzati dell'aria sulle alte montagne Atti R. Accad. d. sci. di Torino, vol. XVIII, pp. 263-272, 1883, 1 plate.

('84). de Freudenreich, Ed. Des microbes de l'air des montagnes. La semaine médicale, 11 septembre, 1884, pp. 301-302

Relates to scarcity of bacteria in the air at high altitudes. In the summer, in the Bernese Alps, in four places at altitudes varying from 2,000 to 4,000 meters, a total of 2700 litres of air were aspirated without finding any bacteria. The following summer tests were made on a glacier 2,900 meters high and on the summit of a mountain 2,356 meters high, vegetation reaching nearly to the top. On the glacier 2,000 litres of air were aspirated in six portions. Two of the sowings remained sterile. One gave a micrococcus, another B. subtilis, one after more than 15 days gave a torula, and one a mold. "Il nous resterait 2 bactéries pour 2,000 litres, soit un par mètre cube." In Bern the author says he obtains hundreds and thousands of bacteria per cubic meter using the same delicate methods.

The experiments on the mountain yielded 8 bacteria from 2,325 litres of air.

('84). de Freudenreich, Edouard. Recherches sur les organismes vivants de l'air des hautes altitudes. Archiv. des sci. physiques et naturelles, 3e Période, T. douzieme, Genève, 1884, pp 365-387.

Author shows that the purity of the air on mountains is much greater than former writers have supposed.

('84). Miquel, P Des organismes microscopique de l'air de la mer. La semaine médicale, 1884, pp. 90-92.

('84). Hesse, W. Ueber quantitative Bestimmung der in der Luft Enthaltenen Mikroorganismen. Mitth a d. K. Gesundheitsamte, Berlin, vol. II, 1884, pp. 182-207.

('85). Miquel, P. Septième mémoire sur les organismes microscopiques de l'air et des eaux. Annuaire de l'observatoire de Montsouris pour l'an 1885, pp. 467-611.

The part relating to microorganisms in the sea-air begins on p. 514. This subject is also treated in the report of the observatory for 1886, pp. 535-550.

('86). Beumer. Zur Bakteriologie des Bodens. Deutsche med. Wochenschr, Bd. XII, 1886, pp. 464-466

('86). Fischer, B. Bakteriologische Untersuchungen auf einer Reise nach Westindien. Zeitschr f. Hyg, Bd 1, 1886, pp. 421-464.

This paper discusses the microorganisms and spores found in sea-air

('86) Adametz, Leopold. Untersuchungen über die niederen Pilze der Ackerkrume. Inaugural Dissertation, 78 pp, 2 Taf., Leipzig, 1886 Rev in Centralb f. Bakt., 1887, Bd. I, pp. 8-10.

('87). Petri, R. J. Zusammenfassender Bericht über Nachweis und Bestimmung der pflanzlichen Microorganismen in der Luft. Centralb. f. Bakt., Bd. II, 1887, pp. 113-118 und 151-158.

('87). Maggiora, A. Ricerche quantitative sui microorganismi del suolo con speciale riguardo, all'inquinazione del medesimo Giornale della R Accademia de medicina di Torino, 1887, vol. XXXV, Series 3, pp. 153-172.

('87). Fraenkel, Carl. Untersuchungen uber das Vorkommen von Mikroorganismen in verschiedenen Bodenschichten. Zeitschr. f. Hyg, 1887, Bd. II, pp 521-582.

('89) Reimers, John. Ueber den Gehalt des Bodens an Bacterien Zeitschr. f. Hyg., 1889, Bd. VII, pp. 307-346.

('89). Reimers, John Ueber den Gehalt des Bodens an Bacterien. Inaug. Dissert. Jena, 1889, 8vo, 44 pp, Leipzig, Veit u. Comp. Reviewed in Centralb f Bakt, 1 Abt, Bd. X, 1891, p. 489.

"Die Zone dieser plotzlichen Keimverminderung liegt im Jenenser Boden—wie im Berliner—zwischen 1 und 2 metres."

('90) Kramer. See III

('91). Manfredi. See XLIII.

('93). Cristiani, H. Analyse bactériologique de l'air des hauteurs puisé pendant un voyage en ballon Ann. de l'Inst Pasteur, T. VII, pp 665-671.

At elevations above the soil of 1,000 meters and upward the cultures remained sterile although in each case 10 litres of air was allowed to bubble through the culture media. Even at much lower levels the majority of colonies are believed to have come from the earth indirectly, i. e., by way of the balloon.

('93). Duclaux, E. La distribution de la matière organique et des microbes dans le sol. Revue critique. Ann de l'Inst Paseur, T. VII, 1893, pp. 823-833

('97). Moritz, Otto, und Neumann, R. O. Ueber einige bakteriologische Wasseruntersuchungen im Atlantischen Ozean. Centralb. f. Bakt. 2 Abt., Bd. XIII, 1904, pp 481-489.

('99) Levin. Les microbes dans les régions arctiques. Ann de l'Inst Pasteur, T. XIII, 1899, pp. 558-567

The arctic air was tested in twenty places, approximately the same amount being filtered at each place. A total of 21,600 litres of this filtered air yielded three bacterial colonies and a few mold spores The surface waters of the arctic also contain few bacteria. Ninety samples of water were taken from the sea at great depths (1,000 to 3,000 metres). These samples also contained bacteria of several kinds but in small numbers The temperature at this depth is below zero centigrade "Tout un monde de bactéries existe à une température qui descend jusqu'à 2° au dessous de zéro"

('01) Gazert, Hans Bakteriologische Aufgaben der deutschen Sudpolar-Expedition. Petermanns geogr Mitteil., Bd XLVII, 1901, pp 153-155

('01). Belli, C. M Chemische, mikroskopische und bakteriologische Untersuchungen uber den Hagel. Hyg Rdsch., Berlin, Bd. XI, 1901, pp. 1,181-1,187.

('02). Binot, Jean. Étude bactériologique du massif du mont Blanc. Nature, Paris, (1er semest), 1902, pp 350-362, av. fig. C. R. des sé. de l'Acad. des sci, T. CXXXIV, 1902, pp. 673-676.

XLIII. Soil-Organisms; Putrefactive Organisms.

('82). Tyndall. See xlii.

('86). Adametz. See xlii.

('86). Beumer. See xlii.

('87). Fraenkel. See xlii.

('89). Reimers. See xlii.

('91). Berthelot, M., et André, G. Sur l'odeur propre de la terre. C. R. de sc. l'Acad. des sci., Paris, 1891, T. cxii, 598.

('91). Manfredi, Luigi. Sulla contaminazione della superficie stradale nelle grandi città dal punto di vista dell'igiene e dell'ingegneria sanitaria. Recerche e studi fatti con speciale riguardo alla città di Napoli. Atti della R. Accad. delle sci. fis. e mat. di Napoli 2a serie, vol. iv, 1891, appendice, No. 4, pp. 1-79.

('93). Duclaux. See xlii.

('96) Duclaux. See xxviii.

('99) Levin. See xlii.

('01). Smith, R. Greig. Bacteria and the disintegration of cement. Proc. Linn. Soc. of New South Wales, vol. xxvi, for the year 1901, Part 1, Sydney, 1902, pp. 107-117. Also a separate (issued Aug. 13, 1901).

Disintegration not due to the bacteria.

('02). Katayama, T. On the general occurrence of Bacillus methylicus in the soil. Bull. of the College of Agr., Tokyo Imperial Univ., vol. v, No. 2. 1902, pp. 255-258. Also a separate.

('02). Chester, Frederick D. The bacteriological analysis of soils. Proc. 23d Ann. meeting of Soc. for Prom. Agric. Sci., 1902, pp. 173-182. Also a separate

('02). Chester, Frederick D. Bacteria of the soil in their relation to agriculture. Bulletin No. 98, Dept. of Agric. of Pennsylvania, 1902, pp. 88, with plates. A bibliography of 105 titles.

('02). Remy, Th. Bodenbakteriologische Studien. Centralb. Bakt., Abt. 2, Bd. viii, 1902, pp. 657-662, pp. 699-705, pp. 728-735, pp. 761-769.

('04). Chester, Frederick D. Observations on an Important Group of Soil Bacteria. Organisms related to Bacillus subtilis. Fifteenth Annual Report of the Delaware College Agrl. Exp. Sta., for 1903, Newark, Del., U. S. A. With 5 plates. Also a separate, pp. 1-54. Copy of separate received from author October 15, 1904.

XLIV. Vinegar-Bacteria.

('61). Pasteur. Acetic fermentation due to bacteria. Ann. scient. de l'Ecole normale supérieure, 1861.

Not seen.

('68). Pasteur, Louis. Etudes sur le vinaigre, sa fabrication, ses maladies, moyens de les prévenir; nouvelles observations sur la conservation des vins par la chaleur. Paris, 1868. Gauthier-Villars, Imprimeur-Libraire. Victor Masson et Fils, Libraires, pp viii, 119

('86). Brown. See xli.

('86). Brown, A. J. The chemical actions of pure cultivations of Bacterium aceti. Jour. Chem. Soc. Trans., 1886, vol. xlix, London, pp. 172-187.

('87). Brown, A. J. Further notes on the chemical action of Bacterium aceti. Jour. Chem. Soc., London, 1887, vol. li, Transactions, pp. 638-642.

('93). Hansen, Emil Chr. Botanische Untersuchungen über Essigsäurebakterien. Ber. d. deutsch. bot. Gesellsch., Bd. xi, 1893, pp. (69)-(73). General Versammlungs-Heft.

('93). Lafar, F. Physiologische studien uber Essiggärung und Schnell-Essigfabrikation. Centralb. f. Bakt., 1893, Bd. xiii, pp. 684-697. Bibliography of 13 titles.

('—). Hansen, E. C. Recherches sur les bactéries acétifiantes. Compt. rend. d. trav. du Lab. Carlsberg, T. iii, Liv. 3, pp. 182-216.

('94). Hansen, Emil Ch. Recherches sur les bactéries acétifiantes. Ann. de micrographie, T. vi, 1894, No. 8, pp. 385-395; No. 9, pp. 441-470. Also a separate, pp. 41. 14 text figs.

('95). Lafar. Physiologische studien über Essiggärung und Schnellessigfabrikation. Centralb. f. Bakt., 2 Abt., Bd. 1, 1895, pp. 129-150.

('97). Lafar. See iii.

('98). Beyerinck, M. W. Ueber die Arten der Essigbakterien. Centralb. f Bakt., 2 Abt., Bd. iv, 1898, pp. 209-216.

('00). Hansen, Emil Ch. Recherches sur les bactéries acétifiantes. (Troisième mémoire.) C. R. des travaux du laboratoire de Carlsberg, T. v, 1re Livraison, 1900, pp. 39-46, 1 fig. Also a separate. Copenhagen, 1900.

XLV. Silage-Bacteria, Fermentation of Tobacco, of Indigo, Retting of Flax, of Sisal Hemp, Etc., Softening of Pickles, Sauerkraut, Etc.

(See also XX and XLIV.)

('87). Alvarez, E. Sur un nouveau microbe, déterminant la fermentation indigotique et la production de l'indigo bleu. C. R. des sc. de l'Acad. des sci., Paris, 1887, T. cv, pp. 286-289.

('89). Burrill, T. J. The biology of ensilage. Bull. Ag. Exp. St. Univ. of Ill., 1889, No. vii, pp. 177-194.

('91). Albert, Friedrich. Untersuchungen über Grünpressfutter. Jahrb. d. deutsch. Landwirtsch.-Gesellsch., Bd. vi, Tl. 1, pp. 149-250, Berlin, 1891.

This author says bacteria exert a preponderant influence on the course of the fermentation.

('91). Suchsland, Emil. Ueber Tabaksfermentation. Ber. d. deutsch. bot. Gesselsch., Bd. ix, Berlin, 1891, pp. 79-81.

('94). van Lookeren-Campagne, C. J. Bericht über Indigo-Untersuchungen, ausgeführt an der Versuchs-Station zu Klatten auf Java. D. landw. Vers.-Stat., 1894, Bd. xliii, pp. 401-426.

('95) WINOGRADSKY, SERGIUS. Sur le rouissage du lin et son agent microbien C. R. des sé. de l'Acad. des sci., Paris, 1895. T cxxi, pp. 742-745

A résumé of the principal results of work done by Fribes in Winogradsky's laboratory.

1. Stems sterilized under water by a short heating at 100°, repeated three days in succession, or by one heating at 115° for fifteen minutes, did not become retted

2. Sterilized flax immersed in water and inoculated with any one of the 10 aerobic and anaerobic organisms, first isolated from the macerations by means of gelatin plates, did not undergo, even after several months, a commencement of retting, nor was any liberation of gas noticed. On the contrary—

3. If into tubes of water containing sterilized flax a small bit of straw of unsterilized flax was thrown a very active fermentation commenced at the end of 12 to 15 hours, and at the end of two or three days the retting was completed

The specific organism was obtained for study from successive cultures upon steam-sterilized flax, protected from the air by immersion in deep tubes full of water, whose surface was covered by a layer of oil After a long enough series of re-sowing under these same conditions, the microscopic study of these cultures has removed all doubt about the agent of this fermentation. It is found almost pure in the interior of the stem, and Fribes has succeeded in isolating it in a completely pure state by cultivating it, in the absence of air, upon slices of cooked potato rubbed with chalk. It is a bacillus relatively large, forming spores in the terminal swellings (tadpole form). In the young state its rods are from 10 to 15 μ long, with a thickness of 0.8 μ; often one finds articulated filaments much longer; they become later a little thicker (1 μ), and form then ovoid swellings 3 μ long by 2 μ thick, the ovoid spore which is formed there is 1.8 μ by 1.2 μ. Sterilized flax was retted in pure cultures of this bacillus and, after undergoing the successive operations of grinding (dressing) peeling and combing, yielded a fine silky flax of light color but a little too much retted and without consistence.

In a general study of the bacteria of retting, Fribes discovered the following facts

1. The bacillus ferments glucose, cane-sugar, milk-sugar, and starch, but only when the liquid contains peptone. With ammonia as the only source of nitrogen, the bacillus is absolutely void of action on these eminently fermentable substances.

2. Pectic matters, pectine, or pectic acid, extracted from flax, pears, carrots, white turnips, pure as they can be prepared, are decomposed, in presence of an ammonium salt as the sole nitrogenous food, with an extraordinary facility.

3. Cellulose, under the form of Swedish filter paper, or as an amorphous precipitate, can absolutely not be attacked by this bacillus. Gum arabic is not fermented.

4. Vegetable substance, from flax, white turnips, extracted cold by pure water and water slightly acid and alkaline, and submitted to fermentation by this bacillus, loses the greater part of the substances which are estimated as pectic matter; also the loss of weight of the fermented substance corresponds sensibly to the content in pectic matter of the unfermented substance.

As a result of these studies Winogradsky concludes that the retting of flax may be considered as a pectic fermentation in the micro-biological sense of the word, of which the bacillus described is the specific agent.

('96). TOLOMEI, GIULIO. Ueber die Fermentation der Oliven und die Oxydation des Olivenöles. Atti R. Acad. dei Lincei Roma, se. v, Rendiconti, Classe sci. fis., matem. e nat., vol. v, Feb. 16, 1896, pp. 122-129.

Due to an enzyme. Not bacterial.

('96). VAN LOOKEREN-CAMPAGNE, C. J., U. VAN DER VEEN, P. J. Ueber Indigobildung aus Pflanzen der Gattung "Indigofera." D. landw. Vers-Stat., 1896, Bd. XLVI, pp. 249-258

('97). CONRAD, EUGEN. Bakteriologische und chemische studien über Sauerkrautgährung. Arch. f. Hyg., Bd. XXIX, 1897, pp. 56-95 See also Zeitschr. f. Spiritusindustrie, XX Jahrg., 1897, No 23, p. 188; No. 24, pp. 200-201.

The fermentation of the "Weisskraut" is attributed to Bacterium brassicæ acidæ, Lehm. & Conrad, nearly related to Bacillus coli. This organism is motile, grows aerobically and anaerobically, produces acids, carbon dioxide, hydrogen and marsh gas Gelatin is not liquefied. The surface colonies are gray white to gray yellow on gelatin and agar, and are bright yellow on potato It ferments maltose, lactose and dextrose. Most of the acid is lactic acid.

('98). BRÉAUDAT, L. Sur le mode de formation de l'indigo dans les procédés d'extraction industriels. Fonctions disastasiques des plantes indigofères. C. R. des sé. de l'Acad. des sci., Paris, 1898. T. CXXVII, pp. 769-771.

('98). MOLISCH. Ueber die sogenannte Indigogährung und neue Indigopflanzen. Sitzungsber. d. k. Akad. der Wiss. in Wien, July, 1898. Title only.

('98). PRESCOTT AND UNDERWOOD. See XX.

('99). BRÉAUDAT. Nouvelles recherches sur les fonctions diastasiques des plantes indigofères. C. R. des sé. de l'Acad. des sci., Paris, T. CXXVIII, 1899, pp. 1,478-1,480.

('99) VERNHOUT, J. H. Onderzoek over bacteriën bij de fermentatie der tabak. Mededeelingen uit s'Lands Plantentuin, XXXIV, Batavia, G. Kolff & Co., 1899, p. 49, 2 plates.

The fermentation of tobacco is ascribed to Bacillus tabaci-fermentationis Vernhout.

('99). LOEW, OSCAR. Curing and fermentation of cigar leaf tobacco. U. S Dept. of Agric., Report No. 59, Div. Veg. Phys. & Path., 34 pp.

('00). LOEW, OSCAR. Sind Bakterien die Ursache der Tabakfermentation? Centralb. f. Bakt., 2 Abt., Bd VI, 1900, pp. 108-112

The fermentation is strictly enzymic, and bacteria are not concerned in it.

('00) LOEW, OSCAR. Physiological Studies of Connecticut leaf tobacco. U. S. Dept. of Agric., Div. of Veg. Phys. & Path., 1900. Report 65. 57 pp.

('00). BEIJERINCK. Verdere onderzoekingen over de indigovorming uit weedl (Isatis tinctoria). Proc. K. Akad. Wetensch Amsterdam, Deel IX, June 30, 1900, pp 74-90. Abstr. in Bot. Zeitung, 2 Abt, vol. 58, 1900, col. 188-189

The production of indigo blue is due to the action of the enzyme isatase upon isatan Isatan occurs in the protoplasm; isatase occurs in the chromatophores. In the living cell the author thinks reactions are prevented by the acidity of the cell-sap. He says: "The action of isatase upon isatan is possible only in neutral or amphoteric and very weakly acid solutions." A temperature of 48° to 50° C. is the optimum for this action."

('01). LOEW, OSCAR. Catalase, a new enzym of general occurrence, with special reference to the tobacco plant. U. S Dept. of Agric. Rep. 69, Div Veg. Phys. & Path., 1901, 47 pp.

('01). RUSSELL, H. L., AND BABCOCK, S. M Concerning the theories of silage formation. Science, n s., vol. XIII, p. 328, 1901.

The conclusion reached is that bacteria do not play any very considerable rôle in the fermentation of silage.

('01). PREYER, AXEL. Ueber Kakaofermentation. Tropenpflanzer, Berlin, Bd. v, 1901, pp. 157-173.

('01). SCHULTE IM HOFE, A. Zur Kakao-Fermentation. Tropenpflanzer, Berlin, 1901, Bd. v, pp. 225-227.

('02) Babcock, S. M., und Russell, H. L. Die bei der Herstellung von Gärfutter (Silage) wirkenden Ursachen. Centralb. f Bakt., Abt. 2, Bd ix, 1902, pp. 81-88.

('02) Hauman, L. Étude microbiologique et chimique du rouissage aérobie du lin. Ann. de l'Inst. Pasteur, T. xvi, 1902, pp. 379-385

('02). Bail, Oskar. Untersuchung einiger bei der Verwesung pflanzlicher Stoffe thätiger Sprosspilze. Centralb. f. Bakt., Abt. 2, Bd. viii, 1902, pp 567-584.

('03). Wehmer, C. Die Sauerkrautgärung. Centralb. f. Bakt., 2 Abt., 1903, x Bd., pp. 625-629.

('04). Stoermer, K. Ueber die Wasserröste des Flachses. Centralblatt f. Bakt, 2 Abt., Bd. xiii, No. 1-3, Sept., 1904, pp. 35-45. Continued.

('04). Beijerinck, M. W, and van Delden, A. "On the bacteria which are active in flax-retting." Koninklijke Akademie van Wetenschappen te Amsterdam Proceedings of the Meeting of Jan. 30, 1904. Pub. Feb. 25, 1904, pp. 462-481, 1 plate. Also a separate.

('04). Omélianski, W. Die histologischen und chemischen Veranderungen der Leinstengel unter Einwirkung der Mikroben der Pektin- und Cellulosegarung. Centralb. f. Bakt., 2 Abt., xii Bd., 1904, pp. 33-43, 1 plate.

('04). Wehmer, C. Die Sauerkrautgaerung. Bericht des V Internationalen Kongresses f. angewandte Chemie zu Berlin, 1903. Sekt VI, Bd. iii, Berlin, 1904, p. 712. Also a separate, 5 pp.

Obtains results decidedly different from Conrad. The ordinary sauerkraut fermentation is a mixed fermentation. Gas-development is due to a bottom yeast; acid production to a non-liquefying, non-motile, non-gas-forming, lactic acid bacterium.

XLVI. Bacteria in Water and Ice; Dung-Bacteria.

(See also XXXIII and XXXIV.)

('70). Warming. See xii.

('86). Fraenkel, Carl. Ueber den Bakteriengehalt des Eises. Zeitschr. f. Hyg., Bd. i, 1886, pp. 302-314.

('87). Macé. Sur quelques bactéries des eaux de boisson. Ann. d'hyg. publ. et de méd. lég., avril, 1887. 3d series, T. xvii, pp. 354-357.

('87). Prudden, T. Mitchell. On Bacteria in ice and their relation to disease, with special reference to the ice supply of New York City. Med. Record, March 26 and April 8, 1887, Nos. 13 and 14; also a separate, 61 pp Reviewed in Centralb. f. Bakt, Bd. i, 1887, pp. 650-652, and Ann. de l'Inst. Pasteur, T. i, 1887, pp. 409-410.

Dr. Prudden tested the resistance of various bacteria to prolonged cold, in blocks of ice and to repeated freezings and thawings. Proteus vulgaris and Bacillus prodigiosus did not grow after 51 days freezing. A slender liquefying bacillus from Croton water was killed in seven days. The following withstood freezing: Staphylococcus pyogenes (66 days), a fluorescent bacillus from ice (77 days); bacillus of typhoid fever (123 days) cultures made at intervals showed less and less living, but all were not destroyed. Repeated freezings and thawings were more fatal to the typhoid bacillus than a constant low temperature. Five freezings and thawings at intervals of three days destroyed this bacillus.

('87). Bordoni-Uffreduzzi, Guido Die biologische Untersuchung des Eises in seiner Beziehung zur offentlichen Gesundheitspflege. Centralb. f. Bakt, 1887, Bd ii, pp. 489-497.

('88). Schmelck, L. Eine Gletscherbakterie. Centralb. f. Bakt., 1888, Bd. iv, pp. 545-547.

A green fluorescent organism was the commonest form. This was a short rod which liquefied gelatin.

('91). Viron, L Du rôle des Schizophytes dans les réactions qui se passent dans les eaux distillées. Jour. de Pharm. et de Chim., 1891, T. xxiii, series 5, pp. 586-593.

('91). Nordtmeyer. See xvii.

('94). Laser. See xx.

('94). Houston. See xvii.

('94). Frankland, P., and Mrs. P. Micro-organisms in water. London, 1884, pp. xi, 532.

('95). Sedgwick und Prescott. On the influence of variations in the composition of nutrient gelatin upon the development of water bacteria. Am. Pub. Health Asso., vol. xx, 1895, pp. 450-458. Rev. in Centralb. f. Bakt., xix Bd., 1896, p. 222.

('95). Rabinowitsch. See xxxiv.

('95). Smith, Theobald. Notes on bacillus coli communis and related forms; together with some suggestions concerning the bacteriological examination of drinking water. The Amer. Journal of the Med. Sci., Sept., 1895. Also a separate, pp. 20.

('95). Smith, Theobald. Ueber den Nachweis des Bacillus coli communis im Wasser. Centralb. f. Bakt., xviii Bd., 1895, pp. 494-495.

('95). Severin, S. A. Die im Miste vorkommenden Bakterien und deren physiologische Rolle bei der Zersetzung derselben. Centralb. f. Bakt., 2 Abt, Bd. i, 1895, pp. 97-114 and 799-817.

('97). Hesse, Friedr. Ueber die Verwendung von Nähragar-Agar zu Wasseruntersuchungen Centralb. f. Bakt., xxi Bd, 1897, pp. 932-937.

Finds agar-media better than gelatin on account of number of liquefying organisms constantly present in water.

('97). Kern, Heinrich. Beitrag zur Kenntniss der im Darme und Magen der Vögel vorkommenden Bacterien. Arb. a. d. bact. Inst. d tech. Hochschule zu Karlsruhe, Bd. i, Heft iv, 1897, pp. 379-532.

Many bacteria are described at length.

('98). Ward, H. Marshall. Some Thames Bacteria Annals of Botany, vol. xii, 1898, pp. 287-322 Two double plates in color.

This paper treats of (1) A short colorless bacterium forming stearine-like colonies: type of Bacterium ureae (Jaksch); (2) A colorless capsuled coccus or bacterium; (3) rose-pink Micrococcus: type of M. carneus (Zimm.); (4) A pseudo-bacillus.

('99). Kasansky. See xxxiii.

('99) Fuller, George W, and Johnson, George A. On the differentiation and classification of water bacteria. Jour. Exp. Med., vol. iv, 1899, pp 609-626; also a separate.

('00). Kohlbrugge, J. H. F Vibrion-Studien. i. Die Ubiquitat choleraahnlicher Wasservibrionen. Centralb. f. Bakt., xxviii Bd., 1900, pp. 721-726. ii. Panmorphismus und erbliche Variationen. Ibid, pp. 833-842.

('00) Sedgwick and Winslow. See xxxiii.

('01). Park See xxxiii.

('01). Horrocks, W. H. An introduction to the bacteriological examination of water. London, J. & A Churchill, 1901, pp. x, 300, 5 plates.

('01) Gage, Stephen De M. Bacteriological Studies at the Lawrence Experiment Station, with special reference to the determination of B. coli 33d An Rep. St. Bd of Health of Mass., for 1901, pp. 307-420. Also a separate, pp. 26

('01) Migula, W. Compendium der bakteriologischen Wasseruntersuchung nebst vollstaendiger Uebersicht der Trinkwasserbakterien. O Nemnich, Wiesbaden, 1901, pp. vii, 440, with 2 plates.

Not seen.

('02). Macfadyen. See xxxiii.

('03) Jordan, Edwin Oakes. The kinds of Bacteria found in river water. Journal of Hygiene, vol. iii, No 1, 1903. Also a separate, pp. 1-27.

('03). Immendorf, H. Ueber Stallmist-Bewahrung (Konservierung) mit Chemischen Mitteln. Berlin, Mitt. d. Landw. Ges., Bd xviii, 1903 pp. 90-101.

('03). Schueder. See xviii.

('03). Winslow and Nibecker. See xvii.

('04) Gage and Adams See xvi.

('04). Stokes. See xviii.

XLVII. Milk-Bacteria; Butter-Bacteria; Cheese-Bacteria; Meat-Bacteria.

('81) Jalan de la Croix. See xxxviii.

('82) Schmidt-Muehlheim. Untersuchungen uber fadenziehende Milch. Pfluger's Archiv., 1882, Bd. xxvii, pp. 400-510, 1 fig.

('84) Hueppe, Ferdinand. Untersuchungen uber die Zersetzungen der Milch durch Microorganismen. Mitth. a. d. K. Gesundheitsamte, Bd. ii, Berlin, 1884, pp. 309-371.

('89). Menge, Karl. Ueber rothe Milch Centralb. f. Bakt., vi Bd., 1889, pp 596-602.

('89). Baginsky, Adolf. Rote Milch. Deutsche Medizinal-Zeitung, 1889, No. 9, pp. 106-107.

('89) Baginsky, Adolf. Zum Grotenfelt'schen Bacillus der roten Milch. Deutsche mediz. Wochenschrift, 1889, Bd xv, p. 212.

This organism was isolated from feces. It liquefied gelatin slowly and colored milk a dirty red or red-brown.

('91). Conn, H. W Ueber einen bittere Milch erzeugenden Micrococcus. Centralb. f. Bakt., ix Bd., 1891, pp 653-655.

('91). Adametz, L. Untersuchungen uber Bacillus lactis viscosus, einen weitverbreiteten milchwirthschaftlichen Schadling. Berliner landwirthschaftliche Jahrbucher, 1891, Bd. xx, pp. 185-207, 1 plate. Rev. in Centralb f. Bakt., ix Bd., 1891, pp. 698-700.

('92) Nencki, L., et Zawadzki, J. Sur la sterilisation du lait. Arch. des Sci Biol. publiées par L'institut Impérial de Med. Exp. à St. Pétersbourg, T 1, 1892, pp. 371-397.

Contains a bibliography of 50 numbers.

('93) Duclaux, E Sur le rôle protecteur des microbes dans la crême et les fromages. Ann de l'Inst. Pasteur, T. vii, 1893, pp 305-324.

('93). Bleisch, Max. Ueber bittere Milch und die Sterilisierung der Milch durch Erhitzen unter Luftabschluss. Zeitschr. f. Hyg., 1893, Bd. xiii, pp. 81-99.

('94) Bordoni-Uffreduzzi, Guido. Ein Fall von fuchsinähnlicher Bakterienfarbung des Fleisches. Hygien Rundschau., 1894, Bd iv, pp. 12-14.

('94). v. Klecki, Valerian. Ueber einige aus ranziger Butter kultivierte Mikroorganismen. Centralb. f. Bakt., Bd. xv, 1894, pp. 354-362

('94). Leichmann, G. Ueber eine schleimige Garung der Milch. Landw Ver.-Stat., Bd. xliii, 1894, pp. 375-398. Rev. in Centralb f. Bakt., Bd. xvi, 1894, pp. 122-123

('94) Russell, H. L. Outlines of dairy Bacteriology, pp. vi, 186, 1894. Pub by author. Madison, Wisconsin.

('94) Pammel, L. H An aromatic bacillus of cheese (Bacillus aromaticus n sp). Extracts from the Iowa Agricultural Exp. Station, Bull. No 21, 1894, pp. 1-5. Rev. in Centralb. f. Bakt., Bd. xvi, 1894, p. 128

('94). Weigmann, H., und Zirn, Gg. Ueber "seifige" Milch. Centralb. f Bakt., Bd. xv, 1894, pp. 463-470, mit 2 Abbildungen

('94) Duclaux, Emile. Le lait; études chimiques et microbiologiques 2 tirage, augmenté de notes sur le rôle des microbes et sur les phosphates du lait 376 pp., 12mo. Paris, J B. Baillière & fils, 1894

('94). Henrici, H. Beitrag zur Bakterienflora des Kases. Arb. a d bact Inst. d. tech. Hochschule zu Karlsruhe, Bd. 1, Heft 1, 1894, pp 1-110.

('95). Rabinowitsch See xxxiv.

('95). Jolles, Max, u. Winkler, Ferdinand. Bakteriologische Studien uber Margarin und Margarinproductes. Zeitschr f. Hyg., Bd. xx, 1895, pp. 60-108

The bacterial content of margarin products is slight in comparison with that of natural butter.

('95). Conn, H W Bacteria in the dairy vi. Experiments in ripening cream with Bacillus No. 41 7th Ann. Rep. of the Storrs Ag Ex Station for 1894, pp 57-68 Middletown, Conn., 1895

('96) Conn, H. W The relation of pure cultures to the acid, flavor, and aroma of butter. Centralb. f Bakt., 2 Abt., Bd ii, 1896, pp. 409-415.

('97). Conn, H W. Butter aroma. Centralb. f Bakt., 2 Abt., Bd iii, 1897, pp 177-179.

('99) Maaszen, Albert. Fruchtatherbildende Bakterien Arb. a. d k. Gesundheitsamte, Bd xv, Berlin, 1899, pp. 500-513, 3 pl from photomicrographs.

The plates and text deal largely with Bacillus esterificans, B. ester fluorescens and B praepollens. There are numerous references to literature.

('99). Moore, V A., and Ward, A R. An inquiry concerning the source of gas and taint-producing bacteria in cheese curd Bull No 158, Cornell Univ. Agr Exp Station, 1899, pp. 221-237, 1 plate

('99). Ward, Archibald R. Ropiness in milk and cream. Cornell Univ. Agr Exp. Sta. Dairy Div Bul. 105, 1899, pp. 395-412, 4 figs

Ropiness attributed to Bacillus lactisviscosus

('99). Stadler. See xxxviii.

('00). Weber, A. Die Bakterien der sogenannten sterilisirten Milch des Handles ihre biologischen Eigenschaften, etc. Arb a. d. k. Gesundheitsamte, Bd. xvii, 1900, pp 108-155 With bibliography of 225 titles.

('01). Chodat, R., et Hofman-Bang, N. O. Les bactéries lactiques et leur importances dans la maturation du fromage. Ann. de l'Inst. Pasteur, T. xv, 1901, pp. 36-48.

Discusses relation of tyrothrix to ripening of cheese.

('01). Ward, Archibald R. Further observations upon ropiness in milk and cream. Cornell Univ. Agr. Exp. Sta. Dairy Div. Bull. 195, 1901, pp. 29-39, 2 figs.

('01). Park, Wm. Hallock. The great bacterial contamination of the milk of cities. Can it be lessened by the action of Health authorities? The Journ. of Hygiene, vol. 1, 1901, pp. 391-406. See also N. Y. Univ. Bull. of the Med. Sci., vol. 1, 1901, pp. 71-86.

('02). Fischer, Bernhard. Zur Aetiologie der sogenannten Fleischvergiftungen. Zeitschr. f. Hyg., Bd. xxxix, 1902, pp. 447-510, 2 plates. With a bibliography of 31 titles.

('02). Epstein, St. Untersuchungen über die Reifung von Weichkäsen. Arch. f. Hyg., Bd. xliii, 1902, pp. 1-20.

('02). Conn, H. W., and Esten, W. M. The comparative growth of different species of bacteria in normal milk. Fourteenth Ann. Rep. Storrs Agr. Exp. Station, Storrs, Conn., 1901, pp. 13-80. Middletown, Conn., 1902.

Relates to those bacteria occurring naturally in milk and not to those introduced by the bacteriologist.

('02). Gruber, Th. Ueber einen die Milch rosafärbenden Bacillus. Bacillus lactorubefaciens Centralb. f. Bakt., Abt. 2, Bd. viii, 1902, pp. 457-462.

('02). Gruber, Th. Ueber eine in der Milch Rübengeruch und Rübengeschmack erzeugende Bakterie. Molk. Zeitung, Hildesheim, Bd. xvi, 1902, pp. 351-353.

('02). Harding, H. A., und Rogers, L. A. Rostflecken in Cheddarkäse. Centralb. f. Bakt., Abt. 2, Bd viii, 1902, pp 442-443.

Ascribed to Bacillus rudensis.

('02). Richter, Albrecht P. F. Bakterielles Verhalten der Milch bei Boraxzusatz. Arch. f. Hyg., Bd. xliii, 1902, pp. 151-156.

('02). Grimm, Max. Ueber einen neuen aromabildenden Bacillus nebst einigen Bemerkungen uber Reinkulturen für Exportbutter Centralb. f. Bakt., Abt. 2, Bd. viii, 1902, pp. 584-590.

('02). Rosam, A. Ueber Konservierung der Milch mittels Wasserstoffsuperoxyd. Centralb. f. Bakt., Abt. 2, Bd. viii, 1902, pp. 739-744, pp 769-774.

('03). Harrison, F. C., and Cumming, M. The bacterial flora of freshly-drawn milk, Part iv. Journal of Applied Microscopy, vol. vi, 1903. No. 2, p. 2,181. Bibliography of 25 titles.

('03). Swithinbank, Harold, and Newman, George. Bacteriology of Milk. With special chapters by Dr. Newman on the spread of disease by milk and the control of the milk supply. With chromo-lithographs, Woodbury type reproductions of photographs of cultures, and other illustrations of bacteria and of apparatus, and also charts illustrating epidemics. London, 1903, John Murray, pp. xx, 605.

('03). Conn, H. W., and Stocking, W. A., Jr. Comparison of bacteria in strained and unstrained samples of milk. Rep't of Storrs Agr. Exp. Station, Conn., 1902-3, pp. 33-37.

('03). Conn, H. W., and Stocking, W. A., Jr. Series ii. Strained and unstrained milk preserved at 70° and 50°. Rep't of Storrs Agr. Exp. Sta., Conn., 1902-3, pp. 38-51.

('03). Conn, H. W., and Stocking, W. A., Jr. Series iii. Aseptic milk. Rep't of Storrs Agr. Exp. Station, Conn., 1902-3, pp. 52-62.

('03). Conn, H. W., and Esten, W. M. Qualitative analysis of bacteria in market milk. Rep't of Storrs Agr. Exp. Station, Conn., 1902-3, pp. 63-91.

('03). Conn, H. W. Bacteria in freshly drawn milk. Rep't of Storrs Agr. Exp. Station, Conn., 1902-3, pp. 92-98.

('03). Conn, H. W. "The relation of temperature to the keeping property of milk." Storrs Agr. Exp. Station, Storrs, Conn. Bull. 26, Oct., 1903, pp. 3-15.

The author's summary is as follows.

1. Variations in temperature have a surprising influence upon the rate of multiplication of bacteria. At 50° these organisms may multiply only 5-fold in 24 hours, while at 70° they may multiply 750-fold.

2. Temperature has a great influence upon the keeping property of milk. Milk kept at 95° (heat of the cow's body) will curdle in 18 hours, while the same milk kept at 70° will not curdle for 48 hours, and if kept at 50° F. the temperature of an ice-chest, may sometimes keep without curdling for two weeks or more.

3. So far as the keeping property of milk is concerned, the matter of temperature is of more significance than the original contamination of the milk with bacteria.

4. Milk preserved at 50° or lower will keep sweet for a long time, but it becomes filled with bacteria of a more unwholesome type than those that grow at higher temperatures. Old milk is not fit for market, even though it be perfectly sweet.

('03). Wilhelmy. Die Bakterienflora der Fleischextracte und einiger verwandter Präparate. Arb. a. d. Bact. Inst. der techn. Hochschule zu Karlsruhe, iii Bd., i Heft, 1903, pp. 1-42, with 3 plates (18 photomicrographs).

Most of the bacteria exist in form of spores. Twelve new species are described: Micrococcus carniphilus, M. marginatus, Streptococcus debilis, Bacterium flaveum, B. insulosum, Bacillus carniphilus, B. canaliculatus, B. carnis, B. intermittens, B. anthraciformis, B glaciformis, B. micans, B. kaleidoscopicus. B. carniphilus occurs in most meat extracts. Other species found were: Bacterium rusticum Kern, Bacillus mesentericus Flügge, B. vulgatus Flügge, B. cereus Frankland, B. laevis Frankland, and B. vegetus Kern.

XLVIII. Bacteria in Bread.

('85). Laurent, Emile. La bactérie de la fermentation panaire. Bull. de l'Acad. roy. de Belgique, 3 sér., T. x, 1885, pp. 765-775.

('88) Arcangeli, G. Sulla fermentazione panaria. Atti della Società toscana di scienze naturali residente in Pisa. Memorie. Pisa, 1888, vol. ix, pp. 190-211. Bibliog. 20 titles.

('89) Kratschmer und Niemilowicz. Ueber eine eigentümliche Brotkrankheit. Wiener klinische Wochenschrift, 1889, Bd. ii, pp. 593-594.

Authors ascribe the stringy bread to Bacillus mesentericus vulgatus Flügge. They did not determine from what source the bread was infected, but state that the organism will not grow in acid dough or acid bread, but that it grows luxuriantly in feebly alkaline dough or bread.

('89). Peters, W. L. Die Organismen des Sauerteiges und ihre Bedeutung für die Brotgährung. Bot. Zeitung, 1889, Bd. xlvii, col. 405-419, 421-431, 437-449.

('90). Uffelmann, J. Verdorbenes Brot. Centralb f. Bakt., viii Bd., 1890, pp 481-485.

('90). Popoff, M. Sur un bacille anaérobie de la fermentation panaire. Ann. de l'Inst. Pasteur, 1890, T. iv, pp. 674-676.

('94). Lehmann, K. B. Ueber die Sauerteiggärung und die Beziehungen des Bacillus levans zum Bacillus coli communis. Centralb. f. Bakt., Bd. xv, 1894, pp. 350-354.

('94). Waldo, F. J., and Walsh, David. Does baking sterilize bread? The Lancet, London, 1894 (ii), pp. 906-908.

The general conclusion is that baking does not fully sterilize. The authors cultivated numerous kinds of bacteria from the interior of baked loaves.

('97). Vogel, J. Beitrag zur Kenntniss des "fadenziehenden Brotes" Zeitschr. f. Hyg., Bd. xxvi, 1897, pp. 398-416.

('99). Juckenack, Adolf. Beitrag zur Kenntniss des "fadenziehenden Brotes." Zeitschr. f. Untersuch. d. Nahrungs- und Genussmitteln, ii Jahrg., 1899, pp. 786-788.

('00). Thomann, J. Beitrag zur Kenntniss des "fadenziehenden Brotes." Centralb. f. Bakt., 2 Abt., Bd. vi, 1900, pp. 740-743.

Stringy bread was found due to bacteria introduced with the flour. The bacillus isolated by the author out of bread and two kinds of flour is called Bacillus panis viscosi (Vogel). Its cultural characters are given as follows:

It is sporiferous, actively motile, stains by Gram's method, liquefies gelatin rapidly, forms a dry, gray-white growth on agar, spreads widely and is wrinkled and gray-white on potato, grows in grape-sugar bouillon without gas-formation, produces a thick pellicle on peptone bouillon with a clear fluid under it, and grows best at 40° to 42° C.

('01). Duclaux. Pain filant. See Traité, T. iv, pp. 513-515.

('01). Beulshausen, Friedrich. Zur Kenntnis der Ursache des Klebrigwerdens von Brot. Diss. Rostock (Druck v. C Hinstorff), 1901, p. 24.

('02). Michels, Wolfgang. Zur Entstehung des fadenziehenden Brotes. Diss. Kiel. Königsberg i. Pr. (Durck v. Hartung), 1902, p. 15.

('02). Lehmann, K. B. Hygienische Untersuchungen über Mehl und Brot x. Neue Studien uber die Acidität des Brotes, ihre Ursachen und ihre beste Bestimmungs-methode. Arch. Hyg., München, Bd. xliv, 1902, pp. 214-237.

XLIX. Iron-Bacteria.

('88) Winogradsky, S. Ueber Eisenbacterien. Bot. Zeitung, 46 Jahrg., 1888, col 261-270.

('92). Molisch, Hans. Die Pflanze in ihrer Beziehung zum Eisen. Eine physiologische Studie. Jena, 1892, Gustav Fischer, 119 pp., 1 table.

('95). Macallum, A. B. On the distribution of assimilated iron compounds, other than haemoglobin and haematins, in animal and vegetable cells. Quarterly journal of microsc. science, 1895-96, vol. xxxviii, new series, No 150, pp. 175-274, with 3 plates.

The part relating to the bacteria begins on page 254.

('97). Marpmann, G. Bakteriologische Mitteilungen. i. Ueber einen neuen Nahrboden für Bakterien. ii. Ueber ferrophile Bakterien. iii. Ueber den Zusammenhang von pathogenen Bakterien mit Fliegen. Centralb. f. Bakt., xxii Bd., 1897, pp. 122-132.

The new substratum is raw silk. Author has found a bacterium which stores iron in its cell-contents It is non-motile, $2\text{-}3 \times 0.8\text{-}2.0 \mu$, ends rounded, form plump, with black polar chromatophores and intermediate gray granules. Many cells are entirely black and opaque. The pigment is insoluble in alcohol, ether, carbon bisulphid and benzine. It becomes bluish opalescent with ammonia, and bleaches with HCl, giving off hydrogen sulphid On adding ferricyanide of potash after HCl, or with it, the bacteria become an intense blue. On peptone-gelatin the organism was white, but when a trace of iron sulphate was added it became black.

('97). Miyoshi, Manabu. Ueber das massenhafte Vorkommen von Eisenbacterien in den Thermen von Ikao. Journ. of the Coll. of Science, Imperial Univ., Tokyo, Japan, vol. x, Pt. ii, 1897, pp 139-142.

('97). Migula. See iii.

('97). Lafar. See iii.

('04). Schorler, B. Beitrage zur kenntnis der Eisenbakterien. Centralb. f. Bakt., 1904, Bd. xii, pp. 681-695.

L. Sulphur-Bacteria.

('86). Certes and Garrigou. See xxxiv.

('87). Winogradsky, Sergius. Ueber Schwefelbakterien. Bot. Zeitung, 1887, Bd xlv, col. 489, 513, 529, 545, 569, 585, 606. 3 figs.

('88). Winogradsky, S. Sur la morphologie et la physiologie des sulfobactéries. Beitr. z. Morphol. und Physiol. d. Bakterien, fasc. i. Leipzig, 1888

Not seen.

('89) Winogradsky, S. Recherches sur les sulfobactéries. Ann. de l'Inst Pasteur, T. iii, 1889, pp. 49-60.

('89) de Toni and Trevisan. Sulphur bacteria. See Saccardo's Sylloge Fungorum, vol. 8, p. 1,027. Species granula sulphuris secernentes.

('93) Zelinsky, N. D. Ueber Schwefelwasserstoffgarung im Schwarzen Meere und den Limans von Odessa Fortschr d. russ. chem. u. phys. Gesellsch., Bd. xxv, Part v, 1893, pp. 298-303. (In Russian.)

('95). Yégounow, M. Sur les sulfo-bactéries des limans d'Odessa. Archiv. des sci. bio. de l'Inst impérial de méd. expér. de St. Pétersbourg, vol. iii, 1895, pp 381-397. Rev. in Ann. de Micr., T. vii, 1895, pp 281-282.

('95). Beyerinck, M. W. Ueber Spirillum desulfuricans als Ursache von Sulfatreduction. Centralb. f. Bakt., 2 Abt., Bd. i, 1895, pp 49-59 and 104-114.

('97) Migula. See iii.

('97). Lafar. See iii.

('97). Miyoshi, Manabu. Studien uber Schwefelrasenbildung und die Schwefelbacterien der Thermen von Yumoto bei Nikko. Jour. College Sci., Imp. Univ. Tokyo, vol. x, Pt ii, pp 143-173, 1897, 1 plate. Rev in The American Naturalist, vol xxxii, 1898, pp 456-457

('01). Conn. See iii.

('02). Nathansohn, Alexander. Ueber eine neue Gruppe von Schwefelbacterien und ihre Stoffwechsel. Mitt. a. d. zool. Stat Neapel., Bd. xv, 1902, pp. 655-680.

('03). Hinze, G Thiophysa volutans, ein neues Schwefel-Bacterium. Ber. der d. bot. Gesellsch., Bd. xxi, Hft. 6, July, 28, 1903, pp. 309-316.

('03). van Delden. See xxiv

LI. Bacteria in Prehistoric Times.

('79). van Tieghem, Ph. Sur la fermentation butyrique (Bacillus amylobacter) à l'époque de la houille. C. R. des sé. de l'Acad. des sci., Paris, 1879, T lxxxix, pp. 1,102-1,104.

('95). Renault, B. Sur quelques bactéries des temps primaires. Bull du Museum d'histoire naturelle, Paris, année 1895, T 1, No. 4, pp. 168-172, 4 figs.

('95). Renault, B. Sur quelques Bactéries due Dinantien (Culm). C. R des sé. de l'Acad des Sci, Paris, T. cxx, 1895, pp. 162-164.

('95). Renault, B. Sur quelques micrococcus du Stéphanien, terrain houiller supérieur. C R. des sé. de l'Acad. des sci., Paris, T. cxx. 1895, pp. 217-220.

('95). Renault, B Sur quelques bactéries anciennes. Bull. du Mus. d'Hist nat, Paris 1895, T. 1, pp. 247-252, 6 figs.

('96). Renault, B. Sur quelques bactéries dévoniennes. C R des sé de l'Acad. des sci., Paris, T. cxxii, 1896, pp. 1,226-1,227.

('96). Renault. Houille et Bacteriacées. Soc. d'nat d'Autun, Bull. ix, Autun, 1896, pp. 475-500, 1 pl.

('96). Renault, B Les bactériacées de la houille. C. R. des sé. de l'Acad. des sci., Paris, T. cxxiii, 1896, pp. 953-955.

('96). Renault, B. Les Bactéries dévoniennes et le genre Aporoxylon d'Unger. Bull. du Mus. d'Hist. nat., Paris, 1896, T. 11, pp. 201-203

('96). Renault, B. Notes sur quelques nouvelles bactéries fossiles. Bull. du Mus. d'Hist. nat., Paris, 1896, T. 11, pp. 285-288, 4 figs.

('96). Renault, B. Recherches sur les bactériacées fossiles. Ann. des sci. nat. bot., viii série, T. 11, 1896, pp 275-349, with 46 figures.

('96). Renault, Bernard. Les bactéries dévonienne et le genre Aporoxylon d'Unger. Bull. d. l. Soc. d'Hist nat. d'Autun, T. ix, 1896, pp 139-142, of the Procès-verbaux des sé

('97). Renault, B. Les bactériacées des bogheads. C. R. des sé. de l'Acad. des sci., Paris, T cxxiv, 1897, pp. 1,315-1,318.

('97). Renault, B. Les bactériacées et les bogheads à Pilas. Bull. d. Mus. d'Hist. nat, Paris, T. iii, 1897, pp 33-39, 4 figs.

('97). Renault, B Les bactériacées des bogheads. Bull. d. Mus. d'Hist. nat., Paris, T. iii, 1897, pp. 251-258, 6 figs

('97). Renault, Bernard. Bogheads et bactériacées Soc. d'histoire naturelle d'Autun. x Bulletin, 1897, pp 433-469, 18 text figures

('98). Renault, B. Les micro-organismes des lignites. C. R des sé de l'Acad des sci, Paris, T cxxvi, 1898, pp 1,828-1,831

('98). Renault, B, et Roche, A. Du mode de propagation des bactériacées dans les combustibles fossiles et du rôle qu'elles ont joué dans leur formation. Soc. d'histoire nat. d'Autun, ix Bull., 1898, pp. 133-147, in the Procès-Verbaux d. sé.

('00). Lemière, L Transformation des végétaux en combustibles fossiles. Essai sur le rôle des ferments. Congrès géologique international de 1900, Paris, T. 1, 1901, pp. 502-520.

LII. Preparation of Slides, Cultures, Etc., for Museums, &c.

('80). Kaiser, Eduard. Verfahren zur Herstellung einer tadellosen Glycerin-Gelatin. Bot. Centralb., Bd. 1, 1880, pp. 25-26.

1 gram best French gelatin, 6 grams aq. dest.; soften 2 hours. Add 7 grams of c. p. glycerin and 1 gram of c. p. carbolic acid to each 100 grams of the preceding. Warm and stir 15 minutes. Filter through glass wool previously washed in distilled water.

('83) Grove, W. B. New methods of mounting for the microscope. (Hillhouse's method for glycerine mounting.) Midland Naturalist, vol. vi, 1883, p. 166. Journal of the R. Microscop. Soc., London, August, 1883, p. 599.

According to Hillhouse, as reviewed by Dippel in Botan Centralb., p. 159. Vol. xvi, 1883, glycerin mounts are readily made tight by substituting Canada balsam dissolved in turpentine for ordinary cements. Ring in ordinary way. Hillhouse says that a drop of glycerin on glass can be covered by a drop of balsam, and the latter will spread over it and adhere firmly to glass around it on all sides, inclosing it completely.

('87). Soyka, J. Ueber ein Verfahren, Dauerpräparate von Reinkulturen auf festem Nahrboden herzustellen. Centralb. f. Bakt., 1887, Bd. 1, pp. 542-544.

('88). Jacobi, Ed. Härtung und Farbung von Plattenkulturen. Centralb. f. Bakt., 1888, iii Bd., pp. 536-538.

('88). Soyka, J., und Král, F. Vorschläge und Anleitungen zur Anlegung von bacteriologischen Museen. Zeitschr. f. Hyg, 1888, Bd. iv, pp. 143-150.

('89). Král, Franz Weitere Vorschläge und Anleitungen zur Anlegung von bakteriologischen Museen. Zeitschr. f. Hyg., Bd. v, 1889, pp. 497-505.

('89). Schill. Kleine Beiträge zur bakteriologischen Technik. 1 Konservirung von Platten- und Reagensglaskulturen. 6 Schimmelpilze hindert man im Wachsthum Centralb. f. Bakt., 1889, v Bd., März 1, No. 10, pp. 337-340.

Cultures are covered for 24 hours with a fluid consisting of equal parts of alcohol and glycerin, to which has been added 1 part per 100 of a 1 per cent solution of mercuric chloride. Preparations treated in this way are said to remain unchanged for years.

Camphor is said to hinder the growth of molds without interfering seriously with bacteria.

('92) Dawson, Charles F Eine Methode, Dauerkulturen von Bakterien hermetisch zu verschliessen Centralb. f. Bakt, xii Bd, 1892. pp. 720-721.

('93). Hauser, G. Ueber Verwendung des Formalins zur conservirung von Bacterienculturen. München. med. Wochenschr., 1893, Bd. XL, pp. 567-568. Rev. in Centralb. f. Bakt., Bd. XIV, 1893, p. 290.

Ten or fifteen drops of fresh formalin are put on filter paper and placed under the cover of the Petri-dish culture. This is then exposed to the vapor of formalin in a close room lined with wet filter paper, 15 drops of formalin being placed on cotton and introduced for each 1000 cc. of air space. As the formalin penetrates the deeper layers of gelatin only slowly, a thin layer should be used for liquefying organisms. The formalin should be allowed to act for several days, and be renewed once or twice. The gelatin appears to be permanently disinfected, will not melt at any temperature, and is unchanged in appearance. To have permanent preparations it is only necessary to keep them from drying out.

('93). Hauser, G. Weitere Mitteilungen über Verwendung des Formalins zur conservirung von Bacterienculturen. München. med. Wochenschr., 1893, Bd. XL, pp. 655-656, No. 35. Rev. in Centralb. f. Bakt., Bd. XIV, 1893, pp. 468-469.

Describes a method of fixing and mounting colonies taken from gelatin plate cultures.

('94). Krueckmann, Emil. Eine Methode zur Herstellung bakteriologischer Museen und Konservierung von Bakterien. Centralb. f. Bakt., Bd XV, 1894, pp. 851-857.

Fixes cultures with mercuric chloride, etc., and preserves them in formalin, air-tight, in the dark.

('97). Pakes, W. C. C., and Eyre, J. W. Formalin as a preservative for cultivations of bacteria. Jour. of Path. and Bact., vol IV, 1897, pp. 418-420. Also a separate, 3 pp.

('01). Conn, H. W. How can bacteria be satisfactorily preserved for museum specimens? Science, n. s., vol. XIII, 1901, p. 326.

LIII. Stock-Cultures, How Best Kept; Vitality on Media.

('89). Czaplewski, E. Zur Anlage bakteriologischer Museen. Centralb. f. Bakt., VI Bd., 1889, pp. 409-411.

This method consists in limiting the amount of air which can reach the culture by saturating the upper part of the cotton plug with melted paraffin. The chief objection to it is the increased difficulty of cleaning the discarded tubes.

('98). Lunt. On a convenient method of preserving living pure cultures of water bacteria. Rev. in Centralb. f. Bakt., XXIII Bd., 1898, pp. 795-796.

Certain water-bacteria may be kept alive for two years or more in sterile water, i. e. much longer than in ordinary culture-media.

('00). Bolley, Henry L. The duration of bacterial existence and [in ?] trial environments. Centralb. f. Bakt., 2 Abt., VI Bd., 1900, pp 33-38.

Reports getting a good growth of Bacillus amylovorus and Bact. dianthi in agar and bouillon by transfers from cultures which had been hermetically sealed for 9 years. Tests of the pathogenic power of these cultures appear not to have been made.

('01). Schultz. See VI.

LIV. Color-Charts; Nomenclature of Colors.

('86). Ridgway, Robert. Nomenclature of colors for naturalists. 195 water colors on ten plates, with rules for making the same and a general discussion of colors. Boston, Little, Brown & Co., 1886.

Valuable, but out of print. Especially useful on account of the number of colors. Another edition in preparation.

('94). Saccardo, P. A. Chromotaxia seu nomenclator colorum polyglottus additis speciminibus coloratis ad usum botanicorum et zoologorum. 2d ed. Padua. Typis Seminarii, 1894, 8vo., 22 pp., with two tables containing 50 colors.

A cheap and useful color scheme for botanists.

('95). Color chart under Spectrum, in the Standard Dictionary, Funk and Wagnalls, New York, 1895.

This may be had separately.

('95). Shuttleworth, E. B. Nomenclature of colors for bacteriologists. Jour. Am. Pub. Health Asso., Oct., 1895, Annual vol. XX. pp 403-407.

('98). Prang, Louis. The Prang standard of color. Popular ed. Boston, 1898. Folio.

('—). Chart of Spectrum Scales made from the Bradley colored papers. Milton Bradley Co., Springfield, Mass. A small sheet (about 3¼x7½ inches) with 90 colors. Also a large folded chart (11x28 inches).

Colors are bright, but must be carefully protected from the light.

LV. Photography and Photomicrography.

('77). Koch, Robert. Verfahren zur Untersuchungen, zum Conserviren und Photographiren der Bacterien. Cohn's Beiträge, II Bd., 3 Heft, Breslau, 1877, pp. 399-434, with 24 photomicrographs on 3 plates.

('81). Koch, R. Zur Untersuchung von pathogenen Organismen. Mitth aus dem Kais. Gesundheitsamte, Bd. I, 1881, pp. 1-48.

The paper is illustrated by 84 heliotypes from photomicrographs.

('83). Sternberg, Geo. M. Photomicrographs and how to make them: pp. XV, 204, with twenty plates of photomicrographs. James R. Osgood & Co., Boston, 1883.

('87). Crookshank, Edgar M. Photography of bacteria Illustrated with 86 photographs reproduced in autotype, pp. XIX, 64, London, H. K. Lewis, 1887.

('87). Roux, E. La photographie appliquée à l'étude des microbes. Ann. de l'Inst. Pasteur, T. I, 1887, pp. 209-225.

('88). Zettnow, E. Das Kupfer-Chrom-Filter. Centralb. f. Bakt., 1888, Bd. IV, pp. 51-52.

This light filter is well adapted to photographing bacteria, whether they are stained red, blue or violet.

This filter is made as follows: For use with sunlight, 160 grams copper nitrate and 14 grams pure chromic acid diluted with water to 250 cc. More convenient to prepare and suitable for most purposes in a layer 1 to 2 cm. thick is 175 grams sulphate of copper and 17 grams bichromate of potash dissolved in 1 litre of water. The copper-chrom-filter transmits only a small portion of the spectrum, viz., those yellow-green rays which act most strongly upon erythrosin plates. For the concentrated solution these rays are from wave length 580 to 560: more diluted from 590 to 545.

('88). Neuhauss, R Verschiedenes über Microphotographie. Zeitschr. f. Mikr., Bd. v, pp. 484-486, 1888.

('90). Neuhauss, Richard. Lehrbuch der Mikrophotographie. pp. xi, 273, with 61 wood cuts, 4 autotypes, 2 collotype plates, and 1 photogravure. Braunschweig, Harald Bruhn, 1890.

A very useful book.

('90). Pringle, Andrew. Practical photo-micrography; by the latest methods. The Scovill & Adams Co., New York, 1890, pp. 183, ix, 42 figs. Frontispiece and 6 plates.

('90 to '99). Vogel, H W. Handbuch der Photographie. Vier Theile enthaltend die photographische Chemie, Optik, Praxis und Kunstlehre. Berlin. I Thiel., 1890, pp. xvi, 351, 12 plates; II Theil., 1894, pp. xi, 367; III Theil., Abt. 1, 1897, pp. x, 311, Abt. II, 1899, pp. x, 159.

('99). The photo-miniature. Tennant and Ward, New York. Begun in 1899.

A series of small, inexpensive volumes, by various authors, on various phases of photography. Some of them excellent. About 70 numbers up to beginning of 1905.

('99). Hubbard, J. G. Color screens as applied to photomicrography. Jour. Bost. Soc. Med Sci., vol. III, 1899, pp. 297-301.

('99). Wright, James H. Examples of the application of color screens to photomicrography. Jour. Bost. Soc. Med. Sci., vol. III, 1899, pp. 302-307.

('01). Abney, Sir William de Wiveleslie. A treatise on photography. Tenth ed. thoroughly revised, with 134 illustrations. Longmans, Green & Co., 39 Paternoster Row, London, New York, and Bombay, 1901, pp. xvii, 425.

An excellent book, but subjects treated very briefly.

('02). Walmsley, W. H. The A B C of photomicrography. pp. viii, 155. Tennant and Ward, New York, 1902.

LVI. Methods and Systems of Classification.

(See also III and X.)

('38). Ehrenberg. See v.

('41). Dujardin, Félix. Histoire naturelle des zoophytes, infusoires comprenant la physiologie et la classification. Paris, 1841.

('65). Davaine. See v.

('65-'67). Trécul, A. Urocephalum. C. R. des sé. de l'Acad. des sci., 1865, T. LXI, p. 156 and 432. Ibid. 1867, T. LXV, p. 513.

The form of bacterium with a spore at one end, which is swollen, was called Urocephalum by Trécul.

('72). Cohn. See v.

('79). Trevisan. Introduzzione allo studio die bacteri. Atti d. Inst. Lombardo, 1879.

('80). Winter. See III.

('81) Zopf, W. Ueber den genetischen Zusammenhang von Spaltpilzformen. Monatsbericht d. Königl. preuss. Akad. d. Wissenschaften, Berlin, 1881, pp. 277-284, 1 plate. See also various editions of Zopf's "Spaltpilze."

('85) De Bary. See III.

('85). Kuentsler, J. De la position systématique des bactériacées. Jour. de micr., T. IX, 1885, pp. 295-307.

According to this author the bacteria are of animal origin, the nearest relatives being the Flagellata, especially the astomous forms. There are transition forms.

('86?). Schroeter. See III.

('86). Hueppe, Ferdinand. Die Formen der Bakterien und ihre Beziehungen zu den Gattungen und Arten. With 24 wood-cuts. Wiesbaden, C. W. Kreidel's Verlag, 1886, pp. VIII, 152.

('89). de Toni and Trevisan. Sylloge Schizomycetarum. Forms a portion of vol. VIII of Saccardo's Sylloge Fungorum. Padua, 1889, pp. 923-1,087.

Those volumes of Saccardo's Sylloge Fungorum which are out of print have been reproduced in fac-simile (zincography) by R. Friedlander and Sohn, Berlin.

('90). Messea, Al. Contribuzione allo studio delle ciglia dei batterii e proposta di una classificazione. Revista d'igiene e Sanità Pubblica, Anno 1, 1890, pp. 513-528, 1 plate. Bibliography of 19 titles. Rev. in Centralb. f. Bakt., etc., Bd. IX, 1891, pp. 106-107, and in Baumgarten's Jahresbericht, Bd. VII, p. 344.

The bacteria are classified as Gymnobacteria and Trichobacteria. The latter are subdivided into four groups: Monotricha (one polar flagellum), Lophotricha (a tuft at one pole), Amphitricha (one flagellum at each end), and Peritricha (flagella from various parts of the body). These names are not used in a generic sense.

('92). Ward, H. Marshall. On the characters or marks employed for classifying the Schizomycetes. Annals of Botany, vol. 6, 1892, p. 103.

('94). Migula, W. Ueber ein neues System der Bakterien. Arbeiten aus dem Bakt. Institut der Technische Hochschule zu Karlsruhe, Bd. I, Hft. 2, 1894, pp. 235-238.

('95). Migula, W. Schizomycetes. Engler and Prantl's Die Natürlichen Pflanzenfamilien, Leipzig, Wilhelm Engelmann, 1895.

('95). Fischer, Alfred. Untersuchungen über Bakterien. Jahrb. f. wissensch. Botanik, Bd. XXVII, 1895, Hft. 1, pp. 1-163, 5 plates.

('96). Mez, Carl. Der heutige Stand der bakteriologischen Systematik. Botanisches Centralb., Bd. LXVIII, 1896, pp. 203-211.

('97). Chester, Frederick D. A preliminary arrangement of the species of the genus Bacterium. 9th Ann. Rept. of the Delaware Coll. Agr. Exp. Sta., 1897. Also a separate, pp. 93.

('97). Migula. See III.

('97). Fischer. See III.

('00). Migula. See III.

('02). Thaxter. See x.

('03). Kendall, Arthur I. A proposed classification and method of graphical tabulation of the characters of bacteria. Proc. Amer. Pub. Health Asso, Thirtieth annual meeting, held at New Orleans, La., Dec., 1902, vol. XXVIII, pp. 481-493. Also a separate, pp. 3-15. Pub. 1903.

('03). Ford, William W. The classification and distribution of the intestinal bacteria in man. Studies from the Royal Victoria Hospital, Montreal, vol. 1, No. 5 (Pathology II), 1903, pp. 3-95 3 tables. Also a separate.

('03) Gage, Stephen de M., and Phelps, Earle B. On the classification and identification of bacteria, with a description of the card system in use at the Lawrence Exp. Station for Records of Species (Lawrence, Mass.). Proceedings of thirtieth annual meeting of American Public Health Assn., New Orleans, La., Dec., 1902, vol XXVIII, pp. 494-505 Pub. Columbus, O., 1903 Also a separate, pp. 12-23.

('04). Perkins, Roger G. Bacillus Mucosus Capsulatus. A study of the group and an attempt at classification of the varieties described. Jour of Infectious Diseases, vol. 1, No. 1, 1904, pp. 241-267 Also a separate.

('05). Winslow, C E. A., and Rogers, Anne F. A revision of the Coccaceae. Preliminary communication. From the Biological laboratories of the Massachusetts Institute of Technology. Science, n. s., Vol. XXI, 1905, pp 669-672.

The 445 described forms are reduced to 31 types Five genera are recognized, viz, Diplococcus and Streptococcus, belonging to the sub-family Paracoccaceae, and Micrococcus, Sarcina and Ascococcus, belonging to the subfamily Metacoccaceae.

LVII. Useful Catalogues.

Catalogues and addresses of instrument makers, manufacturers of chemicals, etc.:

Carl Zeiss, Jena.
- (1) Microscopes and microscopical accessories, 32 ed., 1902.
- (2) Photographic objectives and photo-optical appliances, 1901.
- (3) Catalogue of instruments and appliances for projection and photomicrography, fourth ed., 1899.

Ernst Leitz, Wetzlar.
Microscopes and accessory apparatus Cat 39 U S Branch: Wm. Krafft, 80 East 18th st., near Broadway, New York.

Bausch and Lomb, Rochester, N. Y
- (1) Optical apparatus, microscopes, photographic lenses.
- (2) Chemical apparatus, bacteriological apparatus

Eimer and Amend, New York.
Chemical and physical apparatus, 1903. Am. agents for Zeiss

Whitall and Tatum, Philadelphia, New York, and Boston.
Glassware.

F. and M. Lautenschlaeger, Berlin
Cat. No 60 Bacteriologie, Chemie, Asepsis.

Maison Wiesneg (P. Lequeux), 64 Rue Gay-Lussac, Paris.
Catalogue des appareils de bactériologie et d'hygiène.

M. Schanze, Leipzig.
Preisverzeichnis von Mikrotome.

Dr. Hermann Rohrbeck, Berlin.
Brood-ovens, thermo-regulators, etc.

Dr. G Gruebler and Co., Leipzig
Preislisten von Farbstoffe und Reagentien.
Grübler's stains may be obtained in the U. S. from Wm Krafft, 80 East 18th st., near Broadway, New York.

Koenigliche Porzellan-Manufactur, Berlin.
Preis-Verzeichniss, No. v. Gerathschaften zu chemischen und pharmaceutischen Zwecken. 1 Jan., 1899.

Price list of chemical apparatus manufactured and sold by C. Gerhardt, Bonn am Rhein, Germany.

P. J Kipp u. Zohnen, Delft, Netherlands. Makers of the Reinhold-Giltay microtome.

Dr. Rob. Muencke
Catalog über chemische Apparate und Gerathschaften, 1900, Berlin, N W., Luisen-Strasse 58 Preis 5 mark. pp. 600.

Dr. Peters & Rost.
- (1) Preis-Liste über Apparate und Utensilien für elektrochemische und elektrolytische Arbeiten. Liste No. 29 Berlin, 1900, pp. 48
- (2) Preis-Liste über Apparate und Utensilien fur Bakteriologie, Hygiene, Mikroscopie. Reagentien, Farbstoffe, Nahrboden, Reinculturen, mikroscopische Praparate. Liste No. 30. Berlin, 1900, pp. 123.
- (3) Preis-Liste über chemische Apparate und Utensilien für wissenschaftliche und Fabriks-Laboratorien, Chemicalien, Reagentien, Normal-Lösungen. Liste No 28 Berlin, 1902, pp. 534.
- (4) Preis-Liste über physikalische Apparate. Liste No 37. Berlin, 1902, pp. iv, 383.

Max Kaehler and Martini, Berlin
Chemical and Bacteriological Apparatus.

The above five catalogues or their equivalent may now be obtained from the following:

Vereinigte Fabriken für Laboratoriumsbedarf, ges M B H, Chauseestr 3 Berlin, N., formerly Max Kaehler & Martini. Dr. Peters & Rost New York Office: Laboratory and School Supply Co., 20-24 East 20th street, New York

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Bacteriological apparatus, glassware, high-grade glass blowing Very reliable

Greiner & Friedrichs, Stutzerbach, Germany.
Glassware.

C P Goerz.
Price list of double-anastigmat lenses and of the Goerz-Anschutz camera Berlin-Friedenau, and 52 East Union Square, New York.

Folmer and Schwing, 407 Broome street, New York.
High-grade cameras.

Henry Heil Chemical Co., 208-312 S Fourth street, St. Louis, Mo.
Illustrated catalogue and price list of chemical and physical apparatus and instruments for laboratories, chemists, iron and steel works, smelters, assayers, mines, sugar refineries, schools, colleges, universities, etc.

Spencer Lens Co., Buffalo, N Y
Microscopes. Excellent lenses, especially low-power apochromatics and a new 2 mm. oil immersion achromatic.

BECKER, CHRISTIAN.

Price list of balances and weights of precision. Factory: New Rochelle, N. Y.; New York office: 7 Maiden Lane.

THE KNY-SCHEERER CO., 225-233 Fourth avenue, New York.

(1) Illustrated Catalogue of Surgical Instruments. 1902, pp. LVI, and pp. 1,001-1,061, 2,001-2,140, 3,001-3,192, 4,001-4,102, and 5,001-5,180.

(2) Aseptic Surgical Furniture and General Hospital Supplies, 6 ed., 1902, pp. 232. Fully illustrated.

E. H. SARGENT, & CO., 143 and 145 Lake street, Chicago.

Importers and makers of laboratory supplies, price list of apparatus, chemicals, reagents, tissue-stains, microscopical and bacteriological supplies, etc.

JAMES T. DOUGHERTY, 409 and 411 W. Fifty-ninth street, New York.

Scientific apparatus. Sole United States agent for Carl Reichert, Vienna, Austria, microscopes, microtomes, and polarizing apparatus.

THE VOIGTLAENDER & SON OPTICAL CO., 137 W. Twenty-third street, New York.

Collinear and telephoto lenses, porro prism binoculars, etc.

WARNER & SWASEY, Cleveland, Ohio.

Excellent binocular field glasses.

CHARLES J. ROSS CO., 1525 Fairmount avenue, Philadelphia, Pa.

Excellent quality of drawing-board, heavily coated, so as to permit of any number of erasures. Recommended by Dr. Roland Thaxter and by the writer.

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Excellent cameras embodying many convenient, time-saving devices.

STANLEY PHOTOGRAPHIC DRY PLATE CO.

Cheap dry-plates of good quality.

M. A. SEED DRY PLATE CO., St. Louis, Mo.

Standard dry-plates. Non-curling films. Isochromatic, ordinary, and non-halation plates of good quality.

HAMMER DRY PLATE CO., St. Louis, Mo.

Both Seed and Hammer publish interesting little free handbooks on negative making, etc.

G. CRAMER DRY PLATE CO., St. Louis, Mo. New York Depot: 32 East 10th street.

CADETT SPECTRUM DRY PLATE CO., London, England.

THE ILFORD CHROMATIC PLATE CO., London, England.

The 3¼ by 4¼ plates are highly recommended by Dr. Jeffrey for photomicrographic work. They cost 20 cents per dozen, exclusive of duty.

THE EASTMAN KODAK CO., Rochester, N. Y.

Roll and cut films, Kodoid films, non-curling films, solio paper, velox paper, etc.

WILLIS AND CLEMENTS, Philadelphia, Pa.

Platinotype paper.

DR. J. C. MILLER, Denver, Colo.

Blue-print papers of high grade, known as "French Satin, Jr."

G. GENNERT, 24 and 26 East 13th street, New York.

Hauff's Ortol developer, white glass for lantern-slide covers, etc.

MALLINCKRODT CHEMICAL WORKS, St. Louis, Mo., and New York, N. Y.

Photographic chemicals of a high grade.

KEUFFEL & ESSER CO., New York.

127 Fulton street and 42 Ann street. Catalogue and price list of drawing materials and surveying instruments. Drawing paper of any quality desired converted into blue-print paper. Branches in Chicago, St. Louis, and San Francisco.

E. SCHERING, manufacturing chemist, Berlin, Germany.

KRÁL'S LABORATORY, Prag, Austro-Hungary.

Der gegenwärtige Bestand der Král'schen Sammlung von Mikroorganismen, Oct., 1902. Král's Bacteriologisches Laboratorium. Prag, 1. Kleiner Ring 11. Telegramm-Addresse: Král's Laboratorium.

Cultures of several hundred sorts of bacteria and of some fungi may be had from this laboratory. Authors are urged to send their new species to Král.

W. P. Stender, Leipzig, Gerichtsweg 10. Fabrik und Lager von Glasgegenstaenden zur Anfertigung mikroskopischer Praeparate, u. s. w.

EAGLE OXYGEN CO.

(See p. 81.)

DEFENDER PHOTO SUPPLY CO., Rochester, N. Y.

Argo and other photographic papers.

ADDENDA.

Page 46, paragraph *b*, for "fat" read *casein*. The fluid has a soapy feeling, but "saponification" also is probably not the proper term. What actually occurs is a matter for the chemist to determine. The inoculated milk shows no change at first, but gradually becomes intensely alkaline and clears synchronously, without coagulation or precipitation. By addition of acids, or concentrated solutions of sodium chloride, copper sulphate, etc., the transparent fluid may now be filled with a white flocculence, which slowly settles to a bulky caseous precipitate, leaving a supernatant clear, pale whey. It usually requires a month or six weeks for the inoculated milk to become entirely transparent, but a similar transparency may be produced at once in check tubes of milk by adding a few drops of ammonia-water.

On testing cultures grown for a few days in "nitrate bouillon," as described on p. 63, it happens frequently that there is no *nitrite* reaction. It is then necessary to know whether *nitrate* is actually present in the bouillon. Usually cultures of *Bacillus coli*, or some other known nitrate-reducing organism serve this purpose. If such cultures have not been provided, the test for nitrates may be made with diphenylamin dissolved in strong sulphuric acid. On addition of a few drops of this reagent there is an immediate, evanescent, deep-blue reaction if nitrates are present. The reagent is prepared by slowly dropping 90 cc. of c. p. sulphuric acid into 10 cc. of pure water, after which 1 gram of diphenylamin is added, and the solution preserved in a glass-stoppered bottle.

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